3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of carbon tetrachloride. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is

considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of carbon tetrachloride are indicated in Tables 3-1 and 3-2 and Figures 3-1 and 3-2. Because cancer effects could occur at lower exposure levels, Figure 3-2 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10-4 to 10-7), as developed by EPA.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for carbon tetrachloride. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

3.2.1.1 Death

In the past, when industrial and household use of carbon tetrachloride was still common, inhalation exposure to carbon tetrachloride resulted in a considerable number of deaths in humans (e.g., Norwood et al. 1950; Umiker and Pearce 1953). However, quantitative estimates of the exposure levels that caused death are rare. In one case involving inhalation of carbon tetrachloride by an alcoholic, the lethal exposure level was estimated at only 250 ppm for 15 minutes (Norwood et al. 1950). Other workers (nonalcoholics) were exposed at the same level for 4 hours with no significant clinical signs other than slight headache (Norwood et al. 1950).

Lethal inhalation exposure levels in animals depend on exposure duration and species. In mice, the estimated LC_{50} for an 8-hour exposure is 9,500 ppm, with no deaths in 20 animals exposed to 6,300 ppm (Svirbely et al. 1947). In rats, exposure to 7,300 ppm caused no deaths after 1.5 hours, about 50% mortality by 4–6 hours, and 100% mortality by 8 hours (Adams et al. 1952). Exposure to 3,000 ppm for 8–10 hours caused death in 1 of 50 animals. Repeated exposure to 200 ppm 7 hours/day led to increased mortality in rats after approximately 190 days (Adams et al. 1952).

All LOAEL values from each reliable study for death in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.2 Systemic Effects

No studies were located regarding musculoskeletal effects in humans or animals after inhalation exposure to carbon tetrachloride. Studies have been conducted in both humans and animals to evaluate the respiratory, cardiovascular, hematological, hepatic, and renal effects of inhalation exposure to carbon tetrachloride. Gastrointestinal and dermal/ocular effects have been studied in humans but not in animals. These effects are discussed below. The highest NOAEL values and all LOAEL values from each reliable

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
	ACUTE EXPOSURE						
	Death						
1	Human	15 min				250 M (1 alcoholic male died)	Norwood et al. 1950
2	Rat	8-10 hr				3000 (1/50)	Adams et al. 1952
3	Mouse	8 hr				9500 (LC50)	Svirbey et al. 1947
	Systemic						
4	Human	Up to 3 hr	Hepatic		200 M (increased serum bilirubin)		Barnes and Jones 1967
			Renal		200 M (proteinuria)		
5	Human	15 min	Resp			250 M (edema)	Norwood et al. 1950
			Gastro		250 M (nausea)		
			Hepatic			250 M (severe central necrosis)	
			Renal			250 M (oliguria, nephrosis)	
6	Human	70-180 min	Cardio	50 M			Stewart et al. 1961
			Gastro	50 M			
			Hepatic	10 M	50 M (decreased serum iron)		
			Dermal	50 M			

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Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
7	Rat	7 hr	Hepatic	50 M	100 M (fatty degeneration)		Adams et al. 1952
			Renal	100 M			
8	Rat	5-20 d 7 hr/d 5d/wk	Hepatic		10 M (fatty degeneration in 18 M treated 13 times over 17 d)		Adams et al. 1952
9	Rat	1d-15 wk 2d/wk 4hr/d	Hepatic			4800 M (necrosis, fibrosis, cirrhosis, mitogenic and anti-mitogenic activities)	Belyaev et al. 1992
10	Rat	4 d 6hr/d	Hepatic		50 M (steatosis, hydropic degeneration, necrosis, elevated alanine aminotransferase)		David et al 1981
11	Rat	2 wk 5d/wk 8hr/d or 11.5hr/d	Hepatic		100 (Fatty degeneration, increased serum sorbitol dehydrogenase)		Paustenbach et al. 1986b
			Renal	100			
12	Rat	15 min	Hepatic		180 (increased alanine aminotransferase and relative liver weight)		Sakata et al. 1987
13	Rat	6-10 min/d 8 d	Hemato		325 (increased coagulation time)		Vazquez et al. 1990

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Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
	Neurological								
14	Human	15 min			250	(dizziness)			Norwood et al. 1950
15	Human	70-180 min		50					Stewart et al. 1961
16	Rat (albino)	4 hr			611 M	(30% inhibition of response to electrical stimulus)			Frantik et al. 1994
17	Rat	15 min					180	(coma)	Sakata et al. 1987
18	Mouse (H)	2 hr			1370 F	(30% inhibition of response to electrical stimulus)			Frantik et al. 1994
19	Dog	2-10 hr					15000	(depression of central nervous system)	Von Oettingen et al. 1949
	Developmental								
20	Rat	9 d Gd 6-15 7hr/d			330	(decreased fetal body weight and crown to rump length)			Schwetz et al. 1974
	INTERMEDIATE EXPOSURE								
	Death								
21	Monkey	6 wk 5d/wk 8hr/d					80	(1/3)	Prendergast et al. 1967

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Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
22	Rat	173-205 d 5d/wk 7hr/d				200 (9/15 male, 6/15 female)	Adams et al. 1952
23	Gn Pig	180-260d 5d/wk 7hr/d				100 (7/8 males, 4/8 females)	Adams et al. 1952
24	Gn Pig	6 wk 5d/wk 8hr/d				80 (3/15)	Prendergast et al. 1967
25	Gn Pig	90 d cont.				10 (3/15)	Prendergast et al. 1967
	Systemic						
26	Human	8 hr/d intermit.	Gastro		20 (nausea)		Elkins 1942
27	Human	2 mo 8hr/d 5d/wk	Gastro		50 (dyspepsia, nausea)		Kazantzis and Bomford 1960

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Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
28	Monkey	232-277d 5d/wk 7hr/d	Resp	100			Adams et al. 1952
			Cardio	100			
			Gastro	100			
			Hemato	100			
			Musc/skel	100			
			Hepatic	50	100 (slight fatty degeneration)		
			Renal	100			
29	Monkey	10.5 mo 8hr/d 5d/wk	Cardio	200			Smyth et al. 1936
			Hemato	200			
			Hepatic		50 (fatty degeneration)		
			Renal		200 (cloudy swelling of cells in convoluted tubules and loop of Henle)		

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Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
30	Rat	173-205 d 5d/wk 7hr/d	Resp	200					Adams et al. 1952
			Cardio	200					
			Hemato	200					
			Hepatic	5[b]	10	(hepatic fatty degeneration and incr liver wt)	50	(cirrhosis)	
			Renal	100	200	(degeneration of tubular epithelium, elevated blood urea nitrogen, and increased organ weight)			
31	Rat (Fischer- 344)	13 wk 6 hr/d 5 d/wk	Hemato	30	90	(decr hemoglobin and hematocrit)			Japan Bioassay Research Center 1998
			Hepatic		10	(granulation; incr absolute organ wt in F and relative organ wt in M)	270	(fibrosis, cirrhosis; incr relative organ wt in M and absolute organ wt in F)	
			Renal	30 F	10[d] M	(increased absolute and relative organ weight)	810	(hyaline degeneration of glomerulus)	
					90 F	(incr absolute and relative organ wt; vacuolization)			
			Bd Wt	270[d] M	810 M	(decr bd wt)			
				810 F					

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Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
32	Rat	6 wk 5d/wk 8hr/d	Resp	80					Prendergast et al. 1967
			Cardio	80					
			Hemato	80					
			Hepatic				80	(fatty infiltration, cirrhosis)	
			Renal	80					
33	Rat	90 d cont.	Resp	10					Prendergast et al. 1967
			Cardio	10					
			Hemato	10					
			Hepatic	1	10	(fatty degeneration)			
			Renal	10					
34	Rat	10.5 mo 8hr/d 5d/wk	Cardio	400					Smyth et al. 1936
			Hemato	50	100	(hemolysis)			
			Hepatic	50			100	(cirrhosis)	
			Renal		50	(swelling of cells in the convoluted tubules and loop of Henle)			

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Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
35	Mouse	1d-15 wk 2d/wk 4hr/d	Hepatic				4800	(necrosis, fibrosis, cirrhosis, mitogenic and anti-mitogenic activities)	Belyaev et al. 1992
36	Mouse BDF1	13 wk 6 hr/d 5 d/wk	Hemato	270 M 90 F[d]	810 M 270 F[d]	(decr hemoglobin) (decr erythrocyte and hemoglobin)			Japan Bioassay Research Center 1998
			Hepatic	10 F	10 M	(cytological alterations)	30	(hepatic collapse; proliferative ducts in F)	
			Bd Wt	10 M[d] 810 F	30 M				
37	Gn Pig	4-9 mo 5d/wk 7hr/d	Hepatic	5	10	(fatty degeneration)	25	(cirrhosis)	Adams et al. 1952
38	Gn Pig	90 d cont.	Resp	10					Prendergast et al. 1967
			Cardio	10					
			Hemato	10					
			Hepatic	1	10	(fatty degeneration)			
			Renal	10					

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Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
	Immuno/ Lymphoret						
39	Rat (Fischer- 344)	13 wk 6 hr/d 5 d/wk		90 M d 30 F	270 M d 90 F (incr absolute and relative spleen wt)		Japan Bioassay Research Center 1998
	Neurological						
40	Human	>3 mo 8hr/d 5d/wk				80 (narcosis)	Heimann and Ford 1941
41	Human	2 mo 8hr/d 5d/wk			40 (depression)		Kazantzis and Bomford 1960
42	Monkey	232-277d 5d/wk 7hr/d		100			Adams et al. 1952
43	Rat	10.5 mo 8hr/d 5d/wk				50 (sciatic and optic nerve injury)	Smyth et al. 1936
	Reproductive						
44	Rat	10.5 mo 8hr/d 5d/wk		100		200 (decreased litters)	Smyth et al. 1936

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Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
	CHRONIC EXPOSURE								
	Systemic								
45	Rat (Fischer- 344)	104 wk 6 hr/d 5 d/wk	Hemato	5 F	25 F	(decr hemoglobin, hematocrit and lymphocyte; incr leukocyte and segmented neutrophil)			Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998
			Hepatic	5[c]			25	(incr relative liver wt, fibrosis, cirrhosis and deposition of ceroid; incr severity of fatty change and granulation)	
			Renal		5	(severe proteinuria)	25	(incr marked chronic nephropathy)	
			Bd Wt	5	25	(decr bd wt gain)			
46	Mouse (BDF1)	104 wk 6 hr/d 5 d/wk	Hepatic	5			25 F	(thrombus, necrosis)	Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998
							25	(incr liver wt, degeneration, cyst, deposit of ceroid; incr serum enzymes, cholesterol, bilirubin)	
			Renal	5 M	25	(protein casts in males; decr pH and ketone bodies in both sexes, incr urobilinogen and occul blood in females)			
			Bd Wt		25	(decr bd wt gain)			
	Immuno/ Lymphoret								
47	Rat (Fischer- 344)	104 wk 6 hr/d 5 d/wk		25 F	5 M[d]	(incr hemosiderin deposition in spleen)			Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998
					125 F	(incr relative spleen wt)			

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Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
48	Mouse (BDF1)	104 wk 6 hr/d 5 d/wk		5	25 (incr extramedullary hematopoiesis in spleen)		Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998
	Cancer						
49	Rat (Fischer- 344)	104 wk 6 hr/d 5 d/wk				125 (CEL: hepatocellular adenoma in 21/50 M and 40/50 F; hepatocellular carcinoma in 32/50 M and 15/50 F)	Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998

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Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
50	Mouse (BDF1)	104 wk 6 hr/d 5 d/wk				25 M (CEL: adrenal pheochromocytoma in 16/50 males) 125 F (CEL: adrenal pheochromocytoma in 22/49 females) 25 (CEL: hepatocellular adenoma in 27/50 males and 17/50 females; hepatocellular carcinoma in 42/50 males and 33/50 females.	Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998

a The number corresponds to entries in Figure 3-1.

b Used to derive an intermediate inhalation MRL of 0.03 ppm; concentration adjusted for discontinuous exposure by multiplying by 0.21 (7/24 hours/day x 5/7 days/week) and divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability).

c Used to derive a chronic inhalation MRL of 0.03 ppm; concentration adjusted for discontinuous exposure by multiplying by 0.18 (6/24 hours/day x 5/7 days/week) and divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability).

d Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-1. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

Cardio = cardiovascular; CEL = cancer effect level; cont. = continuous; d = day(s); Derm = dermal; F = female; Gastro = gastrointestinal; gd = gestation day; Gn pig = guinea pig; Hemato = hematological; hr = hour(s); incr = increased; intermit. = intermittent; LC50 = lethal concentration, 50% kill; LOAEL = Lowest-observed-adverse-effect level; M = male; min = minute(s); mo = month(s); musc/skel = musculoskeletal; NOAEL = no=observed-adverse-effect-level; ppm = parts per million; Resp = respiratory; wk = week(s).

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Figure 3-1. Levels of Significant Exposure to Carbon Tetrachloride - Inhalation

Acute (≤14 days)

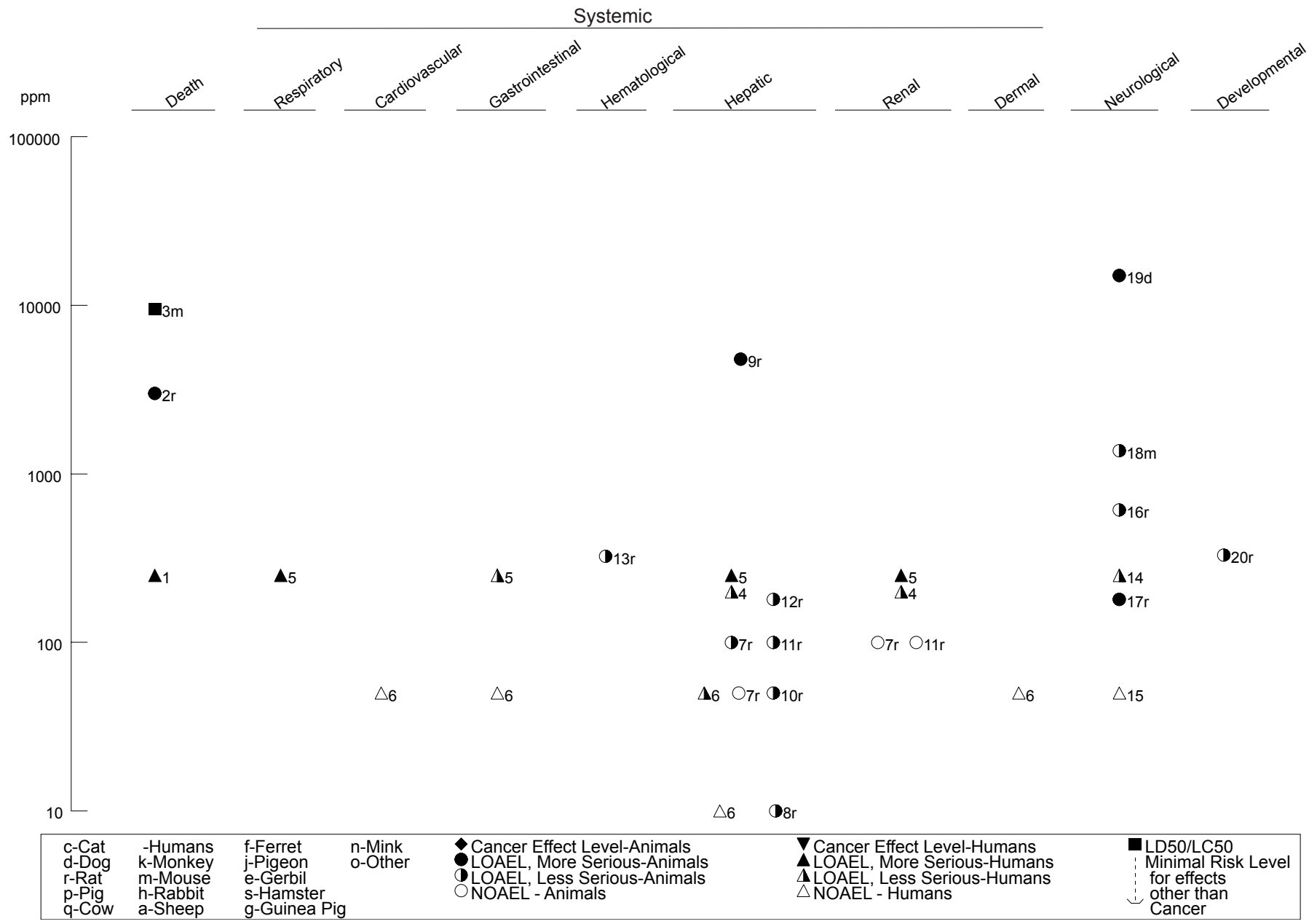

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Figure 3-1. Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (*Continued*)

Intermediate (15-364 days)

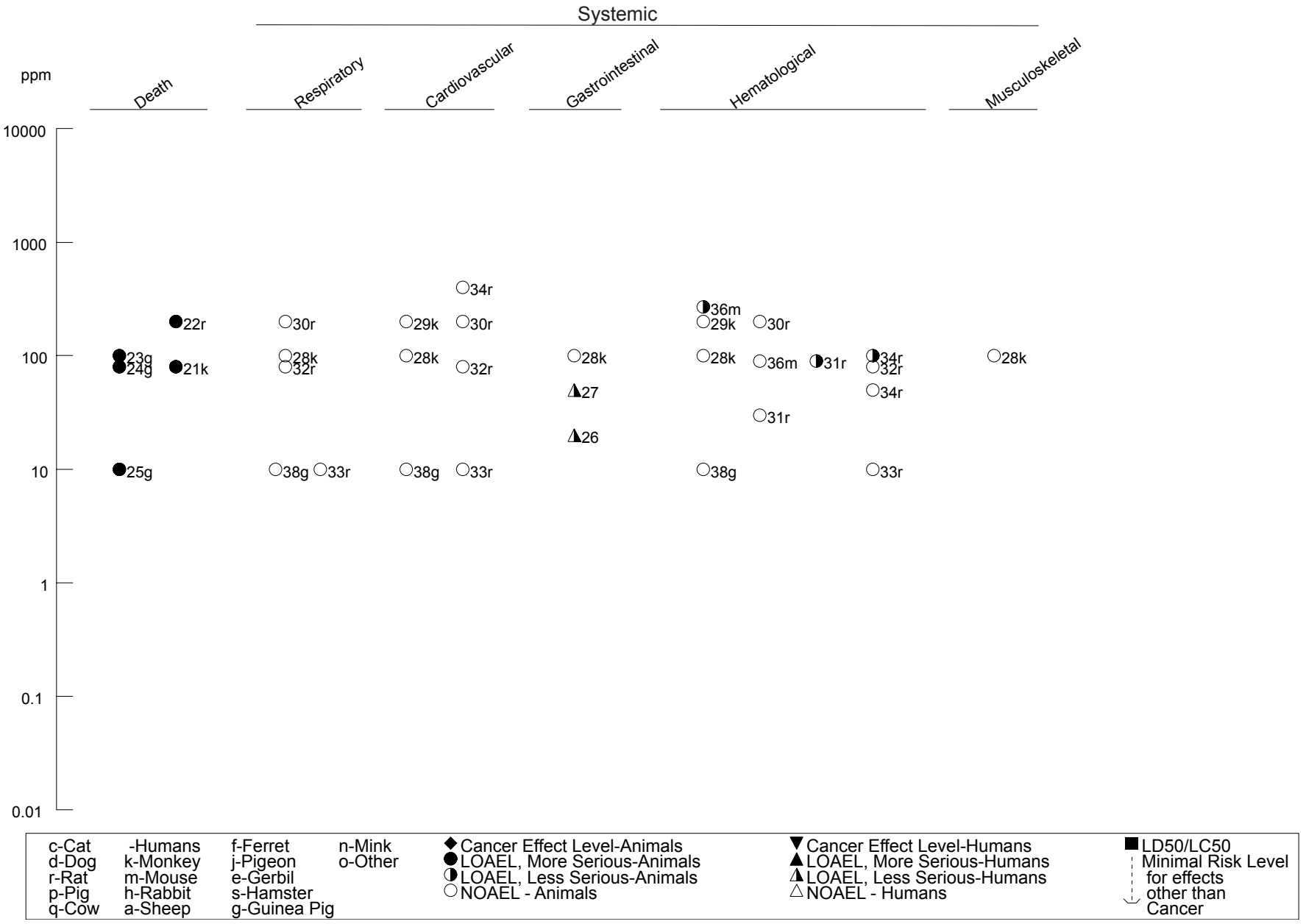

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Figure 3-1. Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (*Continued*)

Intermediate (15-364 days)

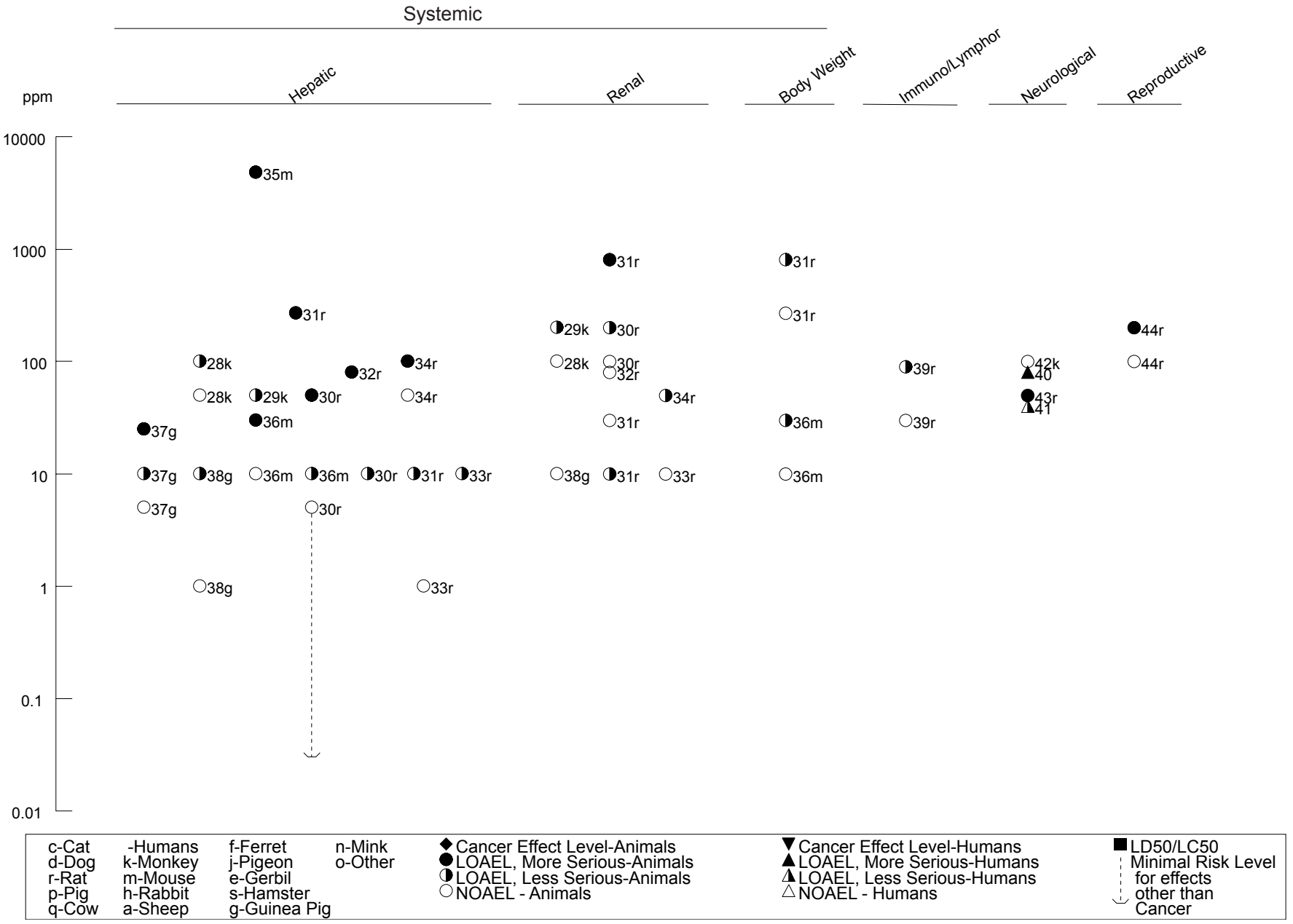

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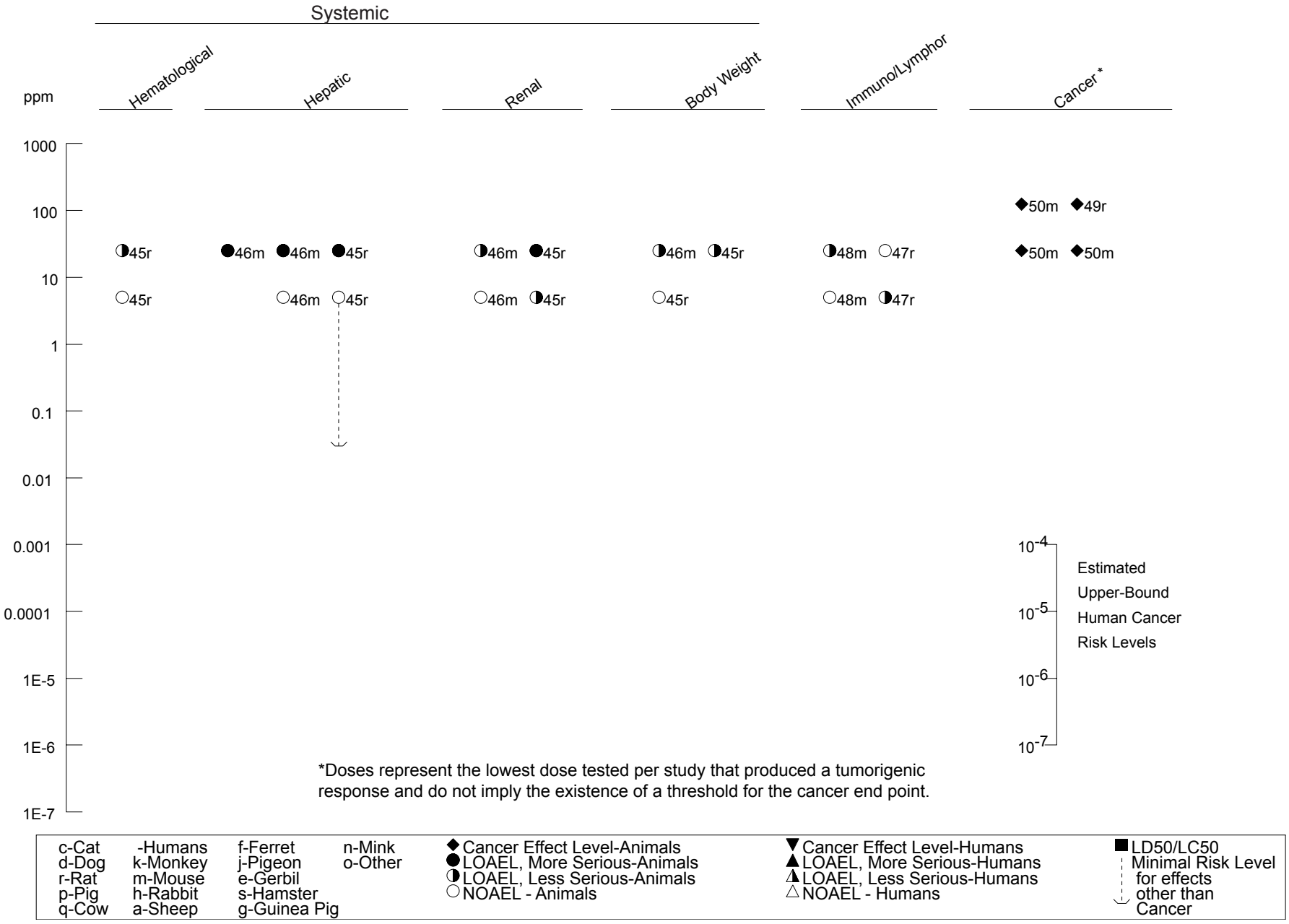
Figure 3-1. Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (*Continued*)

Chronic (≥365 days)



study for systemic effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. Pulmonary edema is a common finding in humans exposed to lethal levels of carbon tetrachloride in air. Thirteen fatal cases were reported following acute inhalation exposure in humans; exposure concentrations were not determined. Marked hemorrhagic congestion and edema were observed in the lungs of all the victims who had been exposed for 1–6 hours (Umiker and Pearce 1953). However, these effects typically did not develop in lung until 8 days after exposure, and appeared to be secondary to severe renal injury rather than to a direct action of carbon tetrachloride on the lung. Lung appearance in the carbon tetrachloride victims was found comparable with that in cases of rapidly developing uremia occasioned by various causes of renal failure. Thus, the progressive uremia, electrolyte retention, and extracellular fluid build-up that accompanies renal failure is a likely principal cause of the observed pulmonary edema.

Lung injury is usually not as prominent an effect in animals exposed to carbon tetrachloride vapors as it is in humans. For example, lung injury was not observed in rats exposed to concentrations of 3,000–19,000 ppm for 7 hours, or in rats and monkeys exposed to 100 ppm for 7 hours/day, 5 days/week for 205 and 232 days, respectively (Adams et al. 1952). After 5 weeks of an exposure sufficient to induce liver cirrhosis and altered vitamin A concentration in several tissues of the rat, wet lung weight was increased by 10%, but lung vitamin A content remained normal (Chapman et al. 1992). As it appears that lung injury is secondary to renal injury, then the absence of lung effects in animals may be because animals are also less susceptible to the renal injury produced by carbon tetrachloride than are humans.

Cardiovascular Effects. Most studies of humans exposed to carbon tetrachloride by inhalation have not detected significant evidence of cardiovascular injury, even at exposure levels sufficient to markedly injure the liver and/or kidney. Changes in blood pressure, heart rate, or right-sided cardiac dilation have sometimes, but not always, been observed (Ashe and Sailer 1942; Guild et al. 1958; Kittleson and Borden 1956; Stewart et al. 1961; Umiker and Pearce 1953), and are probably secondary either to fluid and electrolyte retention resulting from renal toxicity, or to central nervous system effects on the heart or blood vessels. Carbon tetrachloride also may have the potential to induce cardiac arrhythmias by sensitizing the heart to epinephrine, as has been reported for various halogenated hydrocarbon propellants (Reinhardt et al. 1971).

Similarly, except for what are likely secondary effects following acute lethal exposures, significant cardiovascular injury has not accompanied hepato- or renotoxic inhalation exposure to carbon tetrachloride in a variety of experimental animals (Adams et al. 1952; Prendergast et al. 1967; Smyth et al. 1936; von Oettingen et al. 1949).

Gastrointestinal Effects. One of the most common signs of exposure of humans to carbon tetrachloride is dyspepsia, with nausea, vomiting, and gastrointestinal pain (Stewart and Witts 1944). This is often one of the first clinical signs to become apparent following acute exposure (Guild et al. 1958; Norwood et al. 1950), but is also common in persons exposed for months to several years to concentrations as low as approximately 20 ppm (Elkins 1942; Smyth et al. 1936). Exposure levels of approximately 50 ppm do not cause significant dyspepsia if exposure is brief (Stewart et al. 1961), but may lead to nausea if exposure extends for several days (Kazantzis and Bomford 1960). Because inhalation exposure is unlikely to be directly irritating to the gastrointestinal tract, it is probable that these effects are secondary to effects on the autonomic nervous system (Stewart and Witts 1944).

Hematological Effects. Significant effects on the hematological system are not usually observed in humans exposed to carbon tetrachloride by inhalation (Heimann and Ford 1941; Norwood et al. 1950; Smyth et al. 1936). In some cases, moderate elevations in white cell counts are observed, perhaps in response to necrosis in the liver or kidneys. In a few cases, mild anemia is observed (Gray 1947), and may occasionally become severe (Straus 1954). The mechanism underlying anemia is not known, but it might be secondary to internal hemorrhaging as a result of decreased synthesis of clotting factors by the liver or a direct effect on bone marrow cells (Guild et al. 1958; Stevens and Forster 1953; Straus 1954). Since lipid peroxidation caused by carbon tetrachloride also affects calcium sequestration, clotting functions, which are regulated by calcium sequestration would be expected to be impaired, resulting in a tendency for internal hemorrhaging.

Similar observations have been obtained in inhalation studies in animals. Prothrombin time increased and there was lengthened activated partial thromboplastin time in rats dosed 22–40 times with 325 ppm carbon tetrachloride for 10 minutes/day, 5 days/week, indicating defective coagulation in both the extrinsic and intrinsic clotting pathways (Vazquez et al. 1990). No significant effects on hematology were detected in rats, monkeys, or guinea pigs exposed to concentrations of 10–200 ppm, 7 hours/day for periods of time up to 170 days (Adams et al. 1952; Prendergast et al. 1967). Rats exposed for 10 months to 100 ppm suffered some destruction of red blood cells, but this did not result in anemia (Smyth et al. 1936). No evidence of red blood cell hemolysis was observed at 50 ppm. Male and female rats (at

≥90 ppm) and mice (at ≥270 ppm) intermittently exposed to carbon tetrachloride vapor for 13 weeks had decreased hemoglobin levels (Japan Bioassay Research Center 1998); rats also had reduced hematocrit values, whereas female mice had some reduction in erythrocyte counts. Significant reductions in hemoglobin and hematocrit values were observed in female, but not male, rats exposed to carbon tetrachloride vapor for 6 hours/day, 5 days/week for 2 years (Japan Bioassay Research Center 1998).

Hepatic Effects. Carbon tetrachloride has been known for many years to be a powerful hepatotoxic agent in humans and animals. The principal clinical signs of liver injury in humans who inhale carbon tetrachloride are swollen and tender liver, elevated levels of hepatic enzyme (aspartate aminotransferase) in the serum, elevated serum bilirubin levels and the appearance of jaundice, and decreased serum levels of proteins such as albumin and fibrinogen (Ashe and Sailer 1942; McGuire 1932; New et al. 1962; Norwood et al. 1950; Straus 1954). In cases of acute lethal exposures, autopsy generally reveals marked liver necrosis with pronounced steatosis (Jennings 1955; Markham 1967; Smetana 1939), and repeated or chronic exposure leads in some cases to fibrosis and/or cirrhosis (McDermott and Hardy 1963).

Quantitative information on the inhalation exposure levels that cause significant hepatic injury in humans is sparse. Liver necrosis was reported in one fatal case involving an alcoholic who was exposed to 250 ppm carbon tetrachloride for 15 minutes (Norwood et al. 1950). Humans exposed to concentrations of 50 ppm for 70 minutes or 10 ppm for 3 hours showed no measurable change in serum enzyme levels or urinary urobilinogen levels (Stewart et al. 1961). A slight decrease in serum iron levels occurred in two of four subjects exposed to 50 ppm for 1 hour, suggesting to the authors that minimal liver injury had occurred. However, all values were within or close to the normal range of serum iron concentrations, and there were no control subjects. Consequently, it is difficult to judge if the variations observed were treatment-related and whether they were of biological significance. No hepatic effects were observed in humans exposed to average concentrations of 80 ppm for 8 hours/day, 5 days/week for 3 months (Heimann and Ford 1941).

Occasional and slight elevations of serum bilirubin levels were seen in workers exposed for 8 hours/day for several months to many years to carbon tetrachloride concentrations ranging from 10 to 100 ppm, but no other clinical signs of injury were detected (Smyth et al. 1936). Similarly, workers exposed for up to 3 hours/day to carbon tetrachloride concentrations averaging about 200 ppm displayed small increases in serum enzyme levels and serum bilirubin levels, indicative of minimal liver damage (Barnes and Jones 1967). More recently, chronic occupational exposure of 35 male workers to <1 ppm (8 hours/day) of chlorinated solvents, primarily carbon tetrachloride and perchlorethylene, was not correlated with any

significant changes in standard indicators of liver function (e.g., serum levels of protein, albumin, bilirubin, alanine and aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transpeptidase, and cholesterol) (Driscoll et al. 1992). However, when workers were segregated as to having relatively higher or lower exposure, higher exposure was correlated with significantly ($p<0.03–0.05$) lower fasting serum levels of three bile acids (chenodeoxycholate, taurocholate, and total deoxycholate). This effect was in the opposite direction to what might be expected based upon oral animal data and upon serum bile acid increases reported by the same authors for a companion worker population exposed to hexachloro-butadiene or trichloroethylene. Thus, these results should be viewed with caution, especially in view of the low exposure level to carbon tetrachloride and the variable concurrent exposure to several other solvents.

A cross sectional study of hepatic function (serum enzyme levels) was conducted on 135 workers occupationally exposed to carbon tetrachloride and 276 nonexposed controls who were employed in three plants in northern England (Tomenson et al. 1995). Workers were categorized according to their duration of employment (<1 year, 1–5 years, and >5 years), but the serum enzyme results were not presented by estimated duration of exposure, but rather by exposure level. Exposures were estimated from historical personal monitoring data for each job category, and exposure groups were categorized as low (≤1 ppm), medium (1.1–3.9 ppm), or high (≥4.0–11.9 ppm). Alcohol consumption was equivalent among groups. Blood levels of alkaline phosphatase and gamma glutamyl transferase were significantly elevated in exposed workers compared to nonexposed controls. However, the increase did not show a dose-relationship; the differences were only statistically significant for the medium exposure group. None of the exposed subjects had hepatic disease that could be attributed to exposure to carbon tetrachloride.

In animals, the hepatic effects of inhalation exposure to carbon tetrachloride are much the same as in humans: elevated serum enzyme levels, steatosis, and centrilobular necrosis progressing to fibrosis. In rats, exposure to concentrations of 10–100 ppm, 6–7 hours/day generally results in mild to moderate signs of liver injury (fatty degeneration), both after short-term (roughly 2 weeks) and intermediate exposure (3–6 months) (Adams et al. 1952; David et al. 1981; Paustenbach et al. 1986a, 1986b). Four days of exposure at 50 ppm caused elevated serum alanine aminotransferase, altered hepatic glycogen distribution (preferential accumulation in the central and pericentral zones, rather than the uniform distribution observed in controls), steatosis, hydropic degeneration, and necrosis (David et al. 1981). Short-term exposure (15 minutes/day, 2 days/week for 8 weeks) caused fibrosis in rats exposed to 180 ppm (Sakata et al. 1987), whereas a 4-hour exposure to 4,800 ppm induced centrilobular necrosis within 24 hours (Belyaev et al. 1992). With continued biweekly exposures at 4 hours/day, necrotic areas were largely

replaced by hepatocellular proliferation after 2–3 weeks, and then fibrosis and eventually cirrhosis. Liver morphology stabilized after 12–15 weeks, and some reduction in fibrosis was observed 6 weeks after the last exposure. Cirrhosis along with fatty degeneration was observed in rats exposed at 200 or 400 ppm (7 hours/day, 5 days/week for two 2 weeks) (Adams et al. 1952). No acute MRL was established for inhalation exposure to carbon tetrachloride because the value calculated from the most acceptable data would be lower than the intermediate- and chronic-duration MRLs (see Section 2.3), which violates ATSDR policy. The other inhalation MRLs are expected to be protective for acute-duration inhalation exposures.

Mild to moderate liver effects were also found at concentrations of 10–50 ppm after intermediate exposures (6–7 hours/day, 5 days/week) of several months or more (Adams et al. 1952; Bogers et al. 1987; Smyth et al. 1936; Japan Bioassay Research Center 1998). Altered systemic distribution of vitamin A, including significantly reduced hepatic concentrations, has been found to accompany the typically observed liver injury in the rat (Chapman et al. 1992). Although hepatic histopathology was similar in rats and mice exposed for 13 weeks, only rats developed fibrosis and cirrhosis and only mice developed collapse of the liver (Japan Bioassay Research Center 1998). Guinea pigs appear to be somewhat more sensitive to carbon tetrachloride inhalation than rats (Prendergast et al. 1967; Smyth et al. 1936), and monkeys appear to be somewhat less sensitive than guinea pigs and rats (Adams et al. 1952; Prendergast et al. 1967). The basis of these species differences is likely related to differences in hepatic metabolism (see Section 3.4.3). Longer-term exposure to concentrations of 1–5 ppm, 6–7 hours/day, 5 days/week have not been observed to cause any significant changes in liver of rats, monkeys, or guinea pigs (Adams et al. 1952; Prendergast et al. 1967). Based on a NOAEL of 5 ppm (Adams et al. 1952), an intermediate MRL of 0.03 ppm for inhalation exposure to carbon tetrachloride was calculated, as described in the footnote in Table 3-1.

In 2-year inhalation bioassays, concentration-related hepatic effects were observed in rats and in mice following intermittent exposure (6 hours/day, 5 days/week) to carbon tetrachloride vapor (Japan Bioassay Research Center 1998; Nagano et al. 1998). Hepatic changes in rats exposed at 5 ppm, compared to controls, were not statistically significant, but included 2.3-fold increases in the incidences of fatty change, granulation and eosinophilic foci in the liver, a 15% increase in total bilirubin, a 30% increase in serum GOT, a 24% increase in serum GPT in males, and an 18% increase in serum GPT in females. Together with the statistical significance of these changes at ≥25 ppm, the effects at 5 ppm appear to indicate minimal treatment-related hepatic injury. Hepatic lesions at ≥25 ppm included basophilic, eosinophilic, clear and mixed cell foci, deposition of ceroid, fibrosis and cirrhosis, and increased severity of fatty change and granulation. In the parallel assay in mice, there is some uncertainty as to the apparent

NOAEL of 5 ppm because the control values for serum chemistry parameters in males were unusually high compared to the companion subchronic study (no historical control values were available). Hepatic degeneration, thrombus, and deposition of ceroid were evident in both sexes, and hepatic necrosis was found in female mice treated at ≥25 ppm. Statistically significant increases in liver weight and serum enzymes were observed at ≥25 ppm in rats and mice. Based on a NOAEL of 5 ppm in rats (Japan Bioassay Research Center 1998; Nagano et al. 1998), a chronic inhalation MRL of 0.03 was calculated for carbon tetrachloride as described in a footnote in Table 3-1.

Renal Effects. Nephritis and nephrosis are very common effects in humans following inhalation exposure to carbon tetrachloride (Jennings 1955; McGuire 1932; Norwood et al. 1950). The most obvious clinical signs, developing within hours to days after exposure, are oliguria or anuria with resulting edema. In some cases, this leads to generalized uremia, and is frequently accompanied by proteinuria, hemoglobinuria, and glucosuria (Guild et al. 1958; New et al. 1962; Smetana 1939; Umiker and Pearce 1953). In fatal cases, histological examination generally reveals relatively mild degeneration of the kidney (Ashe and Sailer 1942; Gray 1947; Jennings 1955; Norwood et al. 1950). The mechanism of the injury to the kidney is not known, but Sirota (1949) reported that back-diffusion of glomerular filtrate was important in the early stages of oliguria and decreased renal blood flow contributed in the later stages of oliguria following carbon tetrachloride inhalation in humans.

The exposure levels leading to renal damage in humans have not been well defined. An increased incidence of proteinuria was reported in workers exposed to vapor concentrations of around 200 ppm (Barnes and Jones 1967), while no change was observed in urinary properties following inhalation exposure to 50 ppm for 70 minutes or 10 ppm for 3 hours (Stewart et al. 1961).

Threshold concentrations for renal injury in animals exposed by inhalation to carbon tetrachloride are higher than those for hepatic effects. Animals appear to be less sensitive to renal injury than humans, possibly because of species differences in carbon tetrachloride metabolism by the kidney. No evidence of kidney damage was observed in rats, cats, monkeys, or guinea pigs exposed for 6–8 hours/day to concentrations of 10–200 ppm for periods of time from 1 to 90 days (Adams et al. 1952; Bogers et al. 1987; Prendergast et al. 1967). A doubling of vitamin A concentration in the kidneys, along with a 10% increase in wet organ weight, was observed following 5 weeks of intermittent exposure (twice weekly) to an anesthetizing concentration of carbon tetrachloride (Chapman et al. 1992). However, this vitamin A effect may have been secondary to the concurrently induced hepatotoxicity. Slight renal swelling was noted in rats exposed to 50 ppm for 5–10.5 months for 7–8 hours/day, 5 days/week, and in

monkeys exposed to 200 ppm for 10.5 months for 7–8 hours/day, 5 days/week (Adams et al. 1952; Smyth et al. 1936). Renal tubular degeneration was apparent following exposure at 200 ppm for 7 hours/day, 5 days/week (Adams et al. 1952).

Chronic exposure to carbon tetrachloride vapor caused renal effects in rodents, with rats being more sensitive than mice (Japan Bioassay Research Center 1998). Proteinuria and significant progressive glomerulonephrosis were observed in male and female rats exposed to ≥5 ppm for 6 hours/day, 5 days/week for 2 years. Protein casts in the kidney were observed in male and female mice exposed at ≥25 ppm in the same study.

Dermal Effects. Very few reports mention any effect of carbon tetrachloride inhalation on the skin. Inhalation exposure to carbon tetrachloride for several days in the workplace caused a blotchy, macular rash in one man (but not in six others) (McGuire 1932). Similarly, a hemorrhagic rash occurred in a woman exposed to carbon tetrachloride vapors for several days in the workplace (Gordon 1944), and black and blue marks were seen in a patient exposed intermittently to carbon tetrachloride vapors for several years (Straus 1954). Because observations of dermal effects are so sporadic, it is difficult to judge whether these effects are related to carbon tetrachloride exposure, or are incidental. Conceivably, they may have been secondary to reduced synthesis of blood coagulation factors resulting from carbon tetrachloride-induced hepatotoxicity. No animal studies evaluated dermal effects following inhalation exposure.

Body Weight Effects. No human and very few animal reports mention the effect of carbon tetrachloride inhalation on body weight gain. In rodents intermittently exposed to carbon tetrachloride vapor for 6 hours/day, 5 days/week for 13 weeks, reduced body weight gain was observed in male and female rat exposed at 810 ppm and male mice exposed at ≥30 ppm, but not in female mice exposed at concentrations as high as 810 ppm (Japan Bioassay Research Center 1998). However, males and females of both species were affected following exposure to ≥25 ppm in the companion 2-year study (Japan Bioassay Research Center 1998; Nagano et al. 1998).

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans or animals after inhalation exposure to carbon tetrachloride.

3.2.1.4 Neurological Effects

Like many volatile halocarbons and other hydrocarbons, inhalation of carbon tetrachloride leads to rapid depression of the central nervous system. Because of its central nervous system depressant properties, carbon tetrachloride was used briefly as an anesthetic in humans, but its use was discontinued because it was less efficacious and more toxic than other anesthetics available (Hardin 1954; Stevens and Forster 1953). Depending on exposure levels, common signs of central nervous system effects include headache, giddiness, weakness, lethargy, and stupor (Cohen 1957; Stevens and Forster 1953; Stewart and Witts 1944). Effects on vision (restricted peripheral vision, amblyopia) have been observed in some cases (e.g., Johnstone 1948; Smyth et al. 1936; Wirtschafter 1933), but not in others (e.g., Stewart and Witts 1944). In several fatal cases, microscopic examination of brain tissue taken at autopsy revealed focal areas of fatty degeneration and necrosis, usually associated with congestion of cerebral blood vessels (Ashe and Sailer 1942; Cohen 1957; Stevens and Forster 1953).

Exposure levels leading to effects on the central nervous systems of humans are not precisely defined. No symptoms of lightheadedness or nausea were experienced by humans exposed to 50 ppm for 70 minutes or 10 ppm for 3 hours (Stewart et al. 1961), but nausea, headache, and giddiness were found to be common symptoms in workers exposed to carbon tetrachloride for 8 hours/day at concentrations of 20–125 ppm (Elkins 1942; Heimann and Ford 1941; Kazantzis and Bomford 1960). Dizziness has also been reported in humans following short-term exposure (15 minutes) at a higher concentration (250 ppm) (Norwood et al. 1950). This suggests that the threshold for central nervous system effects in humans is, as a conservative estimate, probably in the range of 20–50 ppm for an 8-hour workday.

Central nervous system depression is also observed in animals exposed to carbon tetrachloride vapors. Rats reportedly became inactive within 15 minutes after exposure to a concentration of 180 ppm (Sakata et al. 1987), although when compared with other studies, this concentration appears too low to be capable of inducing such an effect. Drowsiness or stupor occurred in rats exposed for 0.1–8.0 hours to 4,600 ppm, with ataxia and unconsciousness at 12,000 ppm, and death (from respiratory failure) at 19,000 ppm (Adams et al. 1952). Similarly, dogs exposed for 2–10 hours to 15,000 ppm experienced profound depression of the autonomic system, as evidenced by decreases in respiration, reflex activity, body temperature, heart rate, and blood pressure (the latter due to marked vasodilation) (von Oettingen et al. 1949). Exposure of rats, monkeys, or guinea pigs to concentrations of carbon tetrachloride up to 400 ppm, 8 hours/day, 5 days/week for over 10 months did not cause any observable effects on activity, alertness, or appetite, indicating that this level did not cause obvious central nervous system depression in animals (Smyth et al. 1936). However, histological examination of sciatic and optic nerves revealed

degenerative changes in a number of animals exposed to 200–400 ppm, and in a few animals (rats) after exposure to levels as low as 50 ppm under the same exposure schedule. The changes were apparently not severe enough to impair movement or vision. Exposure to 5 ppm carbon tetrachloride vapor for 6 hours/day, 5 days/week for 2 years resulted in decreased absolute brain weights in male, but not female, rats (Japan Bioassay Research Center 1998). However, no histopathology was detected in the brain at that concentration.

The highest NOAEL values and all LOAEL values for each reliable study for neurotoxicity in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to carbon tetrachloride.

In rats that inhaled carbon tetrachloride vapors for three generations, there was a decrease in fertility in animals exposed to concentrations of 200 ppm or higher for 8 hours/day, 5 days/week for 10.5 months (Smyth et al. 1936). Since both sexes were exposed, it was not possible to determine if this was due to effects on males, females, or both. Moderate to marked degeneration of testicular germinal epithelium has been seen in rats exposed repeatedly (7 hours/day, 5 days/week) to 200 ppm or higher for 192 days (Adams et al. 1952). Rats exposed twice weekly for 5 weeks to unspecified anesthetizing concentrations of carbon tetrachloride (and to 0.6 ppm dietary sodium phenobarbital) exhibited a 5% decrease ($p<0.05$) in testes weight (Chapman et al. 1992). Vitamin A levels in the testes were not significantly changed as they were in the liver, kidney, and serum.

Deposition of ceroid was observed in the ovaries of mice that were exposed to 125 ppm of carbon tetrachloride vapor, 6 hours/day, 5 days/week for 2 years (Japan Bioassay Research Center 1998). At 25 ppm, absolute and relative testicular weights were elevated in male mice.

All LOAEL values for each reliable study for reproductive effects in each species and duration category are recorded in Table3-1 and plotted in Figure 3-1.

3.2.1.6 Developmental Effects

No studies were located on developmental effects in humans after known inhalation exposure to carbon tetrachloride. A questionnaire-based study of 3,418 pregnant women in West Germany found no asssociation between probable occupational exposure to carbon tetrachloride (as estimated from a job exposure matrix) and the birth of infants who were small for their gestational age (Seidler et al. 1999).

In rats, inhalation exposure to 330 or 1,000 ppm for 7 hours/day on gestational days 6–15 caused maternal weight loss and clear maternal hepatotoxicity, but no effect on conception, number of implants, or number of resorptions (Schwetz et al. 1974). There were no gross anomalies, although fetal size was somewhat decreased. These data suggest that the fetus is not preferentially sensitive to carbon tetrachloride, and effects of carbon tetrachloride on fetal development and postnatal survival are likely secondary to maternal toxicity.

All LOAEL values for each reliable study for developmental toxicity in each species and duration category are recorded in Table 3-1 and plotted in Figure3-1.

3.2.1.7 Cancer

Two case reports were located that reported the occurrence of liver cancer in humans exposed to carbon tetrachloride vapors, both acutely (Tracey and Sherlock 1968) and for longer periods (Johnstone 1948). In the first case, a 63-year-old male died of hepatocellular carcinoma 7 years after acute intoxication with carbon tetrachloride, although he had a history of moderate alcohol consumption (without demonstrable liver cirrhosis). In the second case, a 30-year-old female died of "liver cancer" after 2–3 years of occupational exposure to carbon tetrachloride that was sufficient to produce signs of central nervous system depression. However, this evidence is much too sparse to establish a cause-and-effect relationship.

A number of epidemiological studies have been conducted to evaluate the association of risk for particular types of cancer and occupational exposure to carbon tetrachloride. Both positive and negative associations have been reported, varying with the target organ. IARC (1999) has noted that few of these studies had definitive evidence of exposure to carbon tetrachloride and that extensive exposure to other possible carcinogenic chemicals could not be excluded. Thus, the associations discussed below are considered suggestive, but are not conclusive.

An analysis of cancer mortality and solvent exposure among a cohort of 6,678 active and retired male workers in the rubber industry found a significant association between age-adjusted exposure to carbon tetrachloride and lymphosarcoma (odds ratio [OR] 4.2, $p<0.05$; based on six cases) and lymphatic leukemia (OR 15.3, $p<0.001$; based on eight exposed cases) (Checkoway et al. 1984; Wilcosky et al. 1984). The authors indicated that confounding nonoccupational or occupational exposures to other solvents could have spuriously caused the association. The same study found no association between exposure to carbon tetrachloride and cancers of the respiratory system. A negative association for lung cancer mortality was also reported in a case-control study of 308 cases (between 1940 and 1981) among 19,603 male employees of a Dow Chemical plant (OR= 0.84, 95% confidence interval [CI]=0.62–1.13) (Bond et al. 1986).

A respective cohort mortality study of 14,457 workers employed at an aircraft maintenance facility for at least 1 year during 1952–1956 included 6,737 workers who had ever been exposed to carbon tetrachloride (Blair et al. 1998). A Poisson regression analysis was performed on cancer incidence data to evaluate the risk from exposure to carbon tetrachloride. Among women, exposure to carbon tetrachloride was associated with an increased risk of mortality from non-Hodgkin's lymphoma (rate ratio [RR] 3.3; 95% CI 0.9–12.7; eight exposed cases) and multiple myeloma (RR 3.3; 95% CI 0.9–12.7; eight exposed cases). Among men, the risks were lower: non-Hodgkin's lymphoma (RR, 1.2; 95% CI, 0.4–3.3; 14 exposed cases) and multiple myeloma (RR, 1.2; 95% CI, 0.4–3.3; 14 exposed cases). No association was found for mortality from breast cancer among women exposed to carbon tetrachloride. Exposure levels were not reported for carbon tetrachloride, and exposures to other solvents were probable.

A case-control study based on death certificates from 24 states evaluated the risk of dying from pancreatic cancer and exposure to several organic solvents (Kernan et al. 1999). The cases were 63,097 individuals who died from pancreatic cancer between 1984 and 1993. The controls were 252,386 persons who died during the same period from causes other than cancer and whose deaths were not caused by pancreatitis or other pancreatic disease. A job-exposure matrix was applied to estimate the intensity and probability of exposure to carbon tetrachloride (none, low, medium, and high) based on occupational and industrial codes. Mortality ORs and 95% CIs were computed to estimate the risk for pancreatic cancer death by occupation, industry, and exposure to various solvents using logistic regression procedures. The risk of pancreatic cancer among deceased individuals was estimated by levels of intensity and probability of exposure (low, medium, and high vs. never exposed). Race- and gender-specific mortality ORs were calculated for black women, black men, white women, and white men. ORs were adjusted for age,

marital status, urban and residential status. No positive associations were found for the intensity of exposure to carbon tetrachloride for any gender/race group. High risks were associated with high probability of exposure to carbon tetrachloride for black men (OR=1.9, 95% CI=1.0–3.7) and white men (OR=1.2, 95% CI=1.0–1.4), but no dose-relationship was observed.

A case-control study evaluated exposures of men in the petrochemical and chemical manufacturing industries to chlorinated aliphatic hydrocarbons, including carbon tetrachloride, as potential risk factors for astrocytic brain tumors (Heineman et al. 1994). A job-exposure matrix was developed by estimating the probability of exposure to carbon tetrachloride and the frequency and magnitude of exposure by industry and job classification, based on likely solvent usage over 6 decades (1920–1980). There were 123 controls and 137 cases identified as having been exposed to carbon tetrachloride at some time. An increase in the incidence of mortality due to astrocytic brain cancer was observed for exposure to carbon tetrachloride. The ORs for the highest-exposure categories were 0.8 (95% CI, 0.4–1.9; 13 exposed cases) for high probability of ever having been exposed, 1.6 (95% CI, 0.8–3.2; 36 exposed cases) for high probability of exposure for more than 21 years, 2.9 (95% CI, 1.2–7.1; 22 exposed cases) for high average intensity of exposure, and 1.6 (95% CI, 0.8–3.2; 24 exposed cases) for high cumulative exposure. According to the authors, the lack of direct information on exposure to solvents was a limitation of the study. In addition, the association of exposure to carbon tetrachloride and brain cancer may have been confounded by exposure to methylene chloride.

A case-control study examined the occupational exposures to some industrial chemicals, including carbon tetrachloride and the relationship to breast cancer in women (Cantor et al. 1995). The probability of exposure was estimated from a job matrix and mortality data were derived from mortality records from 24 states during the period 1984–1989. The study included 33,509 cancer cases and 117,794 controls; of these, 7,211 cases and 29,115 controls had a probability of occupational exposure to carbon tetrachloride. After adjusting for socioeconomic status, there was a suggestive association between exposure probability and level of exposure. At the medium exposure level, the relative risks were 1.15 for Caucasian women and 1.32 for Afro-American women.

A population-based case-control study evaluated the association between occupational exposure to a number of substances and rectal cancer in Montreal, Canada (Dumas et al. 2000). Job history interviews were conducted with 257 individuals with rectal cancer, 1,295 subjects with cancer at other sites, and 533 population controls. There was some association between ever having been exposed to carbon tetrachloride and rectal cancer; the ORs were 2.0 (95% CI, 1.1–3.5; 16 exposed cases) based on cancer

controls and 1.5 (95% CI 0.8–2.9; 16 exposed cases) based on population controls. Occupational exposure to other chemicals is a confounding factor in this study.

Another population-based case-control study of 796 Caucasian patients in Minnesota with renal cell cancer found little or no excess risk in associated with exposure to carbon tetrachloride by males, but a slight nonsignificant excess risk in exposed females (odds ratio 1.88, 95% CI, 0.7–5.0) (Dosemeci et al. 1999). Exposures were estimated on the basis of a job exposure matrix.

Chronic exposure to carbon tetrachloride vapor induced tumors in rats and mice (Japan Bioassay Research Center 1998; Nagano et al. 1998). Following intermittent exposure for 2 years (6 hours/day, 5 days/week), significant increases in the incidences of hepatocellular adenoma and carcinoma were observed in male and female rats exposed at 125 ppm (22.3 ppm, duration adjusted) and in mice exposed at ≥25 ppm (4.5 ppm, duration adjusted). Adrenal pheochromocytomas were also induced in male mice exposed at ≥25 ppm and female mice at 125 ppm.

The carcinogenicity of carbon tetrachloride is currently undergoing reassessment by the EPA under the IRIS program, with the final report scheduled for 2003–2004. As chronic inhalation data were not available for the earlier assessment, the EPA extrapolated oral dose-response data on liver tumor risk to yield estimates of the carcinogenic risk from inhalation exposure to carbon tetrachloride (EPA 1984). Based on the assumption that a 70-kg person breathes 20 m^3/day of air and that 40% of inhaled carbon tetrachloride is absorbed, the calculated upper-bound unit risk (the upper 95% confidence limit on the excess cancer risk associated with lifetime exposure to carbon tetrachloride at a concentration of 1 $\mu g/m^3$) is $1.5x10^{-5}$. Based on this, the concentration of carbon tetrachloride in air corresponding to excess cancer risk levels of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} are 0.001, 0.0001, 0.00001, and 0.000001 ppm, respectively. Because these are upper-bound estimates, the true risk could be lower. These values are displayed in Figure 3-1.

3.2.2 Oral Exposure

3.2.2.1 Death

Ingestion of concentrated solutions of carbon tetrachloride can cause death in humans within hours to days. The principal clinical signs observed in fatal cases include gastrointestinal irritation, central

nervous system depression, and cardiovascular disturbances, with death usually resulting from severe injury to kidney and/or liver (Guild et al. 1958; reviewed in von Oettingen 1964).

There is considerable variation in the doses that have been found to cause lethality, with alcohol ingestion leading to markedly increased risk. Twelve fatalities were reported following oral exposure (Umiker and Pearce 1953). In most cases, about 50–150 mL had been ingested, but one case involved only 5.3 mL (about 121 mg/kg). A review of some of the earlier literature found that ingestion of 14–20 mL (320–450 mg/kg) was fatal in the majority of cases (von Oettingen 1964). In other cases, ingestion of 2.5–15 mL (60–340 mg/kg) as a treatment for hookworm produced death in only a very small number of people out of hundreds of thousands treated, although doses as low as 1.5 mL (40 mg/kg) caused death in a few cases (Lamson et al. 1928). Two fatal cases have been reported in humans dosed with approximately 70 mg/kg (Phelps and Hu 1924).

A single dose oral LD_{50} value of approximately 13,000 mg/kg was reported for mice, and 14 daily doses of 625 mg/kg were lethal for 6 of 20 exposed male mice (Hayes et al. 1986). In rats fed carbon tetrachloride in stock diets or protein-free diets, LD_{50} values of 10,200 or 23,400 mg/kg, respectively were reported (McLean and McLean 1966). The authors attributed the difference in sensitivity in animals in this study to protein depletion, which has reportedly afforded protection against carbon tetrachloride toxicity. This may result from protein depletion-induced reduction in cytochrome P-450 synthesis, with a consequent diminished metabolic activation of carbon tetrachloride to toxic metabolites. In other studies using rats, an LD_{50} value of approximately 7,500 mg/kg was reported (Pound et al. 1973), while 17/20 animals were killed within 14 days of a single oral gavage exposure to 8,000 mg/kg (Thakore and Mehendale 1991). Doses as low as 400 mg/kg have resulted in the death of cats (Chandler and Chopra 1926).

All LOAEL values for each reliable study for death in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

No studies were located regarding dermal, ocular, or musculoskeletal effects in humans or animals after oral exposure to carbon tetrachloride. Studies have been conducted in humans and animals to evaluate the respiratory, cardiovascular, hematological, or hepatic effects. Gastrointestinal and renal effects have been evaluated in humans, and musculoskeletal effects have been noted in animals. These effects are

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Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form
ACUTE EXPOSURE								
Death								
1	Human	Once (C)				40	(lowest quantifiable dose producing death out of 6 cases)	Lamson et al. 1928
2	Human	Once				70	(death in 2/2)	Phelps and Hu 1924
3	Human	Once				120 M	(lowest quantifiable dose producing death out of 12 cases)	Umiker and Pearce 1953
4	Rat	Once (G)				10200	(LD50)	McLean and McLean 1966
5	Rat (Sprague-Dawley)	Once (G)				7500	(LD50)	Pound et al. 1973
6	Rat (Sprague-Dawley)	1 d 1-2x/d (GO)				8000	(death in 17/20)	Thakore and Mehendale 1991
7	Mouse	Once (G)				13000	(LD50)	Hayes et al. 1986
8	Mouse	14 d (G)				625 M	(death in 6/20 males)	Hayes et al. 1986

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
9	Cat	Once (G)					400	(death in 25/36)	Chandler and Chopra 1926
	Systemic								
10	Human	Once	Cardio		2500	(sinus bradycardia and arrhythmia, auricoventricular nodal rhythm, auricular fibrillation)			Conaway and Hoven 1946
			Renal		2500	(increased blood urea nitrogen)			
11	Human	Once (W)	Hepatic		110	(degeneration of hepatocytes)			Docherty and Burgess 1922
			Renal		180	(swelling of proximal convoluted tubules)			
12	Human	Once (W)	Hepatic		90	(slight fatty inflitration)			Docherty and Nicholls 1923
			Renal	90					
13	Human	Once	Renal				2700	(acute tubular necrosis, increased blood urea nitrogen, anuria, proteinuria)	Guild et al. 1958
14	Human	Once	Hepatic				670	(severe necrosis; fatty deposits)	MacMahon and Weiss 1929
			Renal				670	(mild proteinuria, elevated blood urea nitrogen; kidneys swollen, fatty degeneration)	

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Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
15	Human	Once	Gastro		100 (nausea)		Ruprah et al. 1985
16	Human	1-6 d	Resp			120 (substaintial hemorrhagic edema of the lung)	Umiker and Pearce 1953
17	Rat (Fischer- 344)	8-10 d 1x/d (GO)	Hepatic			280 (centrilobular necrosis, increased alkaline phosphatase and 5-nucleotidase)	Blair et al. 1991
18	Rat (Sprague-Dawley)	Once (G)	Hepatic	40 M	80 M (slight vacuolization of some centrilobular hepatocytes)	160 M (increased centrilobular vacuolization with significant necrosis, substantially elevated ornithine carbamyl transferase, sorbitol deydrogenase and alanine aminotranferase)	Bruckner et al. 1986
			Renal	160 M			
19	Rat (Sprague-Dawley)	11 d 9 doses (G)	Hepatic		20 M (limited centrilobular vacuolization, moderately elevated sorbitol deydrogenase, alanine aminotranferase, ornithine carbamyl transferase)	80 M (increased centrilobular vacuolization with some limited necrosis, greatly elevated ornithine carbamyl transferase, sorbitol deydrogenase, alanine aminotranferase)	Bruckner et al. 1986
			Renal	160 M			

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
20	Rat (Sprague-Dawley)	Once (GW)	Hepatic		10 (increased alanin aminotransferase, sorbitol dehydrogenase, ornithine carbamyl transferase; hepatic centrilobular vacuolization)		Kim et al 1990b
21	Rat	Once (F)	Hepatic		20 M (cytoplasmic vacuolization of hepatocytes)		Korsrud et al. 1972
22	Rat	Once (G)	Hepatic		80 M (decreased P-450)	1600 M (centrilobular necrosis)	Matsubara et al. 1983
23	Rat (Fischer- 344)	10 d 1x/d (GO)	Hepatic		5 M[b] (slight vacuolation)		Smialowicz et al. 1991
			Renal	40 M			
24	Rat (Sprague-Dawley)	Once (G)	Renal		4000 M (mitochondrial swelling in cells of proximal tubules)		Striker et al. 1968

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Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
25	Rat (Sprague-Dawley)	1 d 1-2x/d (GO)	Hepatic				480 M	(necrosis, vacuolation; elevated serum levels of aspartate transaminase, alanine transaminase, sorbitol dehydrogenase, decreased liver microsomal cytochrome P-450, aminopyrine demethylase, aniline hydroxylase)	Thakore and Mehendale 1991
26	Rat	Once (GO)	Hepatic	800	1600 3200	(elevated urinary taurine) (lipid vacuoles, 96 hours post-treatment)	3200	(48 hours post-treatment: necrosis lipid vacuolation, inflammation, elevated serum taurine, elevated serum alanine and aspartate amino-transferases, reduced liver taurine)	Waterfield et al. 1991
27	Mouse	Once (G)	Hepatic	10	40	(necrosis)			Eschenbrenner and Miller 1946
28	Mouse (B6C3F1)	14 d 1x/d (GO)	Hepatic		50 F	(increased relative organ weight and SGPT)			Guo et al. 2000
			Bd Wt	1000 F					

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Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to figure	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
29	Mouse (CD-1)	14 d (G)	Hemato		625	(decreased fibrinogen and lymphocyte levels)	Hayes et al. 1986
			Hepatic		625	(increased liver weight, elevated lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase)	
			Renal	2500			
30	Dog	Once (G)	Hepatic		3200	(centrilobular necrosis)	Chandler and Chopra 1926
			Renal		3200	(fatty degeneration)	
31	Dog	Once (G)	Hepatic	160	400	(centrilobular necrosis)	Gardner et al. 1925
			Renal		6400	(fatty accumulation in cortical tubules)	
	Immuno/ Lymphoret						
32	Rat	10 d 1x/d (GO)		160			Smialowicz et al. 1991
33	Mouse (BALB/c)	7 d 1x/d (GO)			500 F	(suppress T-cell activity)	Delaney et al. 1994

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Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
34	Mouse (B6C3F1)	14 d 1x/d (GO)			50 F	(decreased: IgM antibody-forming cell activity per spleen and host resistance to Listeria monocytogenes)			Guo et al. 2000
	Neurological								
35	Human	Once (C)		70					Hall 1921
36	Human	Once (C)		120	300	(drowsiness)			Leach 1922
37	Human	Once					4800	(narcosis)	Stevens and Forster 1953
	Developmental								
38	Rat (Fischer- 344)	Gd 6-15 1x/d (GO)		25 F	50 F	(maternal piloerection and reduced body wt gain during Gd 6-8)	75 F 50 F	(maternal weight loss during Gd 6-8) (total litter resorption in 5/12)	Narotsky et al. 1997a
39	Rat (Fischer- 344)	Gd 6-15 1x/d (G)		25 F	50 F	(maternal piloerection and reduced body wt gain)	50 F	(total litter resorption in 2/14)	Narotsky et al. 1997a
40	Rat	2-3 d (G)					1400	(total litter resorption in 11/29)	Wilson 1954

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to figure	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
	INTERMEDIATE EXPOSURE								
	Systemic								
41	Rat	12 wk (GO)	Hepatic				20	(increased serum enzymes; necrosis; cirrhosis)	Allis et al. 1990
42	Rat	12 wk 5d/wk 1x/d (G)	Hepatic	1[c]	10	(substantially elevated sorbitol dehydrogenase, mild centrilobular vacuolization)	33	(substantially elevated sorbitol dehydrogenase, ornithine carbamyl transferase, alanine aminotransferase, cirrhosis)	Bruckner et al. 1986
			Renal	33					
43	Mouse	5-6 wk (F)	Hepatic	11	19	(increased liver fat and triglycerides)			Alumot et al. 1976
44	Mouse	90 d 5d/wk (G)	Hepatic	1.2	12	(elevated alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase; mild necrosis)			Condie et al. 1986
45	Mouse	120 d (G)	Hepatic	80					Eschenbrenner and Miller 1946

[a] Key to figure

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Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
46	Mouse	90 d (G)	Hemato	1200			Hayes et al. 1986
			Hepatic		12 (centrilobular necrosis, elevated lactate dehydrogenase, alanine aminotransferase, asparte aminotransferase, and alkaline phosphatase)		
			Renal	1200			
	Neurological						
47	Rat	1x/wk 10 wk (G)			290 M (increased serotonin synthesis)		Bengtsson et al. 1987
	Reproductive						
48	Mouse	5-6 wk (F)		36			Alumot et al. 1976
				36			
	Cancer						
49	Mouse	120 d (G)				20 (CEL: hepatoma)	Eschenbrenner and Miller 1946
50	Hamster	30 wk 1x/wk (GO)				120 (CEL: hepatoma)	Della Porta et al. 1961
	CHRONIC EXPOSURE						
	Systemic						
51	Rat	2 yr (F)	Hepatic	11			Alumot et al. 1976
			Renal	11			
	Reproductive						
52	Rat	2 yr (F)		11			Alumot et al. 1976

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Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Cancer						
53	Rat	78 wk 5d/wk (G)				47 (CEL: hepatocellular carcinomas)	NCI 1976

a The number corresponds to entries in Figure 3-2.

b Used to derive an acute oral MRL of 0.05 mg/kg/day; based on treatment of 5 mg/kg/day for 10 consecutive days divided by an uncertainty factor of 90 (3 for the use of a minimal LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability).

c Used to derive an intermediate oral MRL of 0.02 mg/kg/day; dose adjusted for intermittent exposure (5 days/week for 12 weeks) and divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability).

(C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); F = female; (F) = feed; (G) = gavage; (GO) = gavage in oil; Gastro = gastrointestinal; (GW) = gavage in water; Hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mg/kg/day = milligrams per kilograms per day; NOAEL = no-observed-adverse-effect level; Resp = respiratory; (W) = water; wk = week(s); x = time(s); yr = year(s)

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Figure 3-2. Levels of Significant Exposure to Carbon Tetrachloride - Oral

Acute (≤14 days)

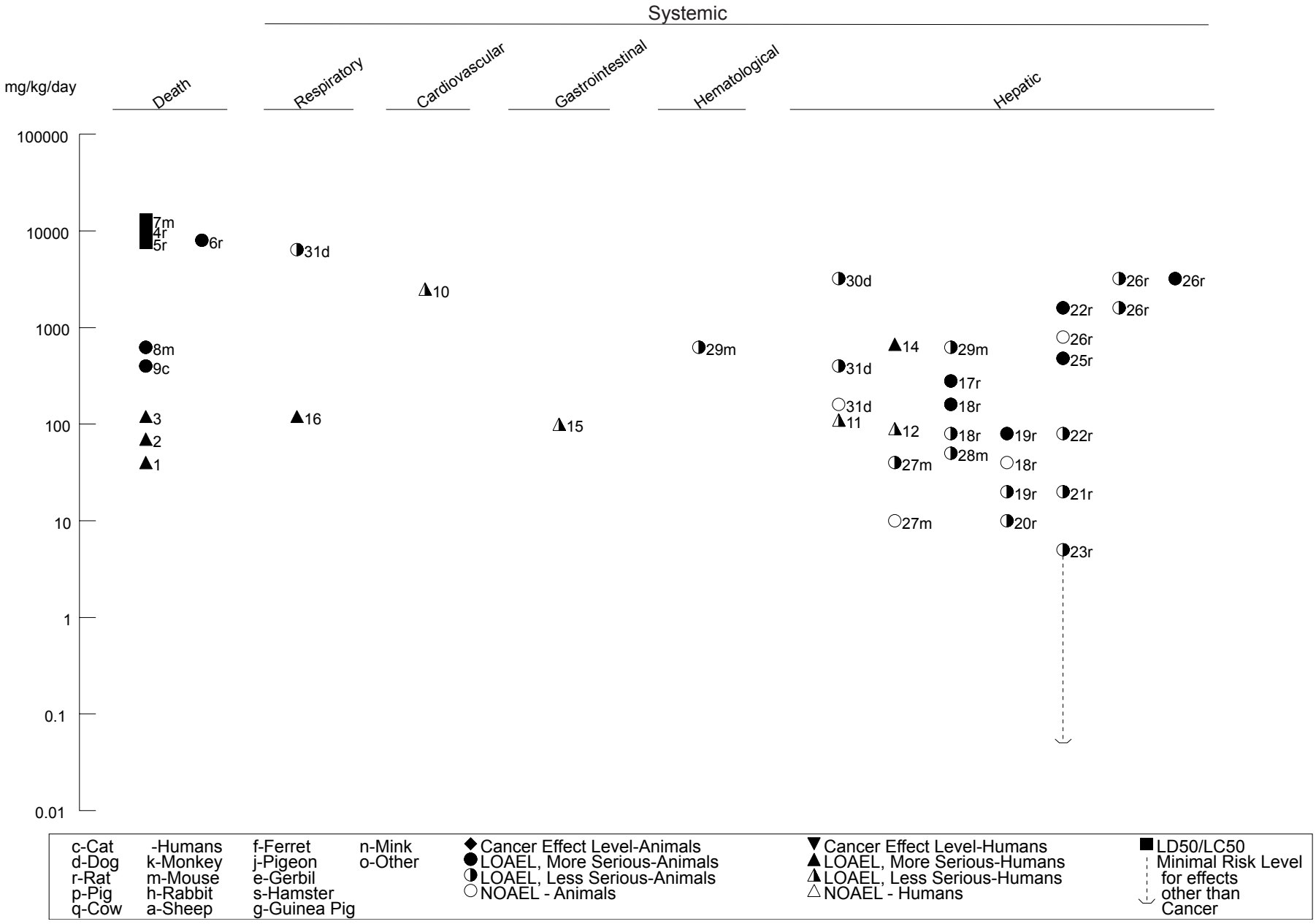

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Figure 3-2. Levels of Significant Exposure to Carbon Tetrachloride - Oral (*Continued*)

Acute (≤14 days)

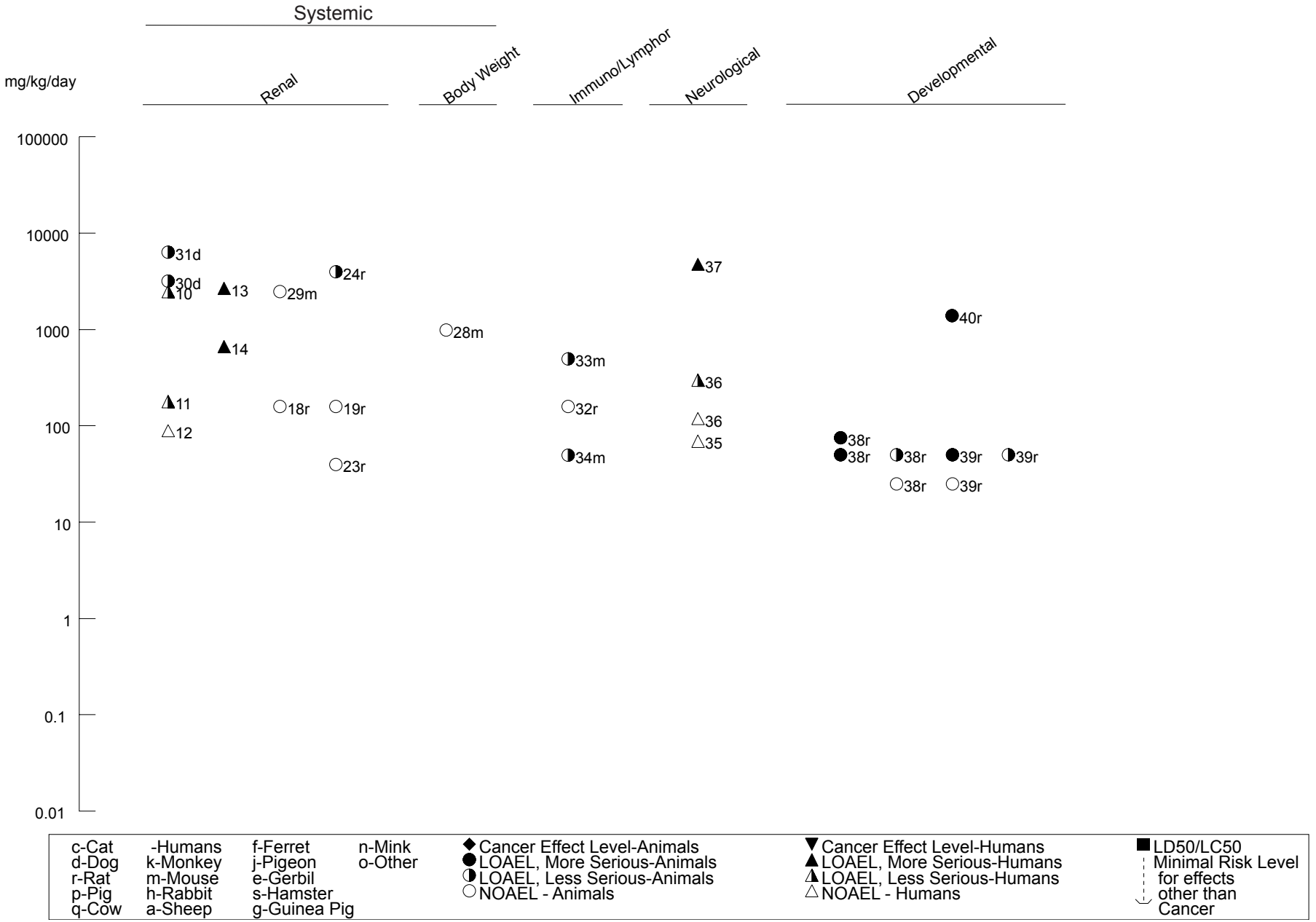

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Figure 3-2. Levels of Significant Exposure to Carbon Tetrachloride - Oral (*Continued*)

Intermediate (15-364 days)

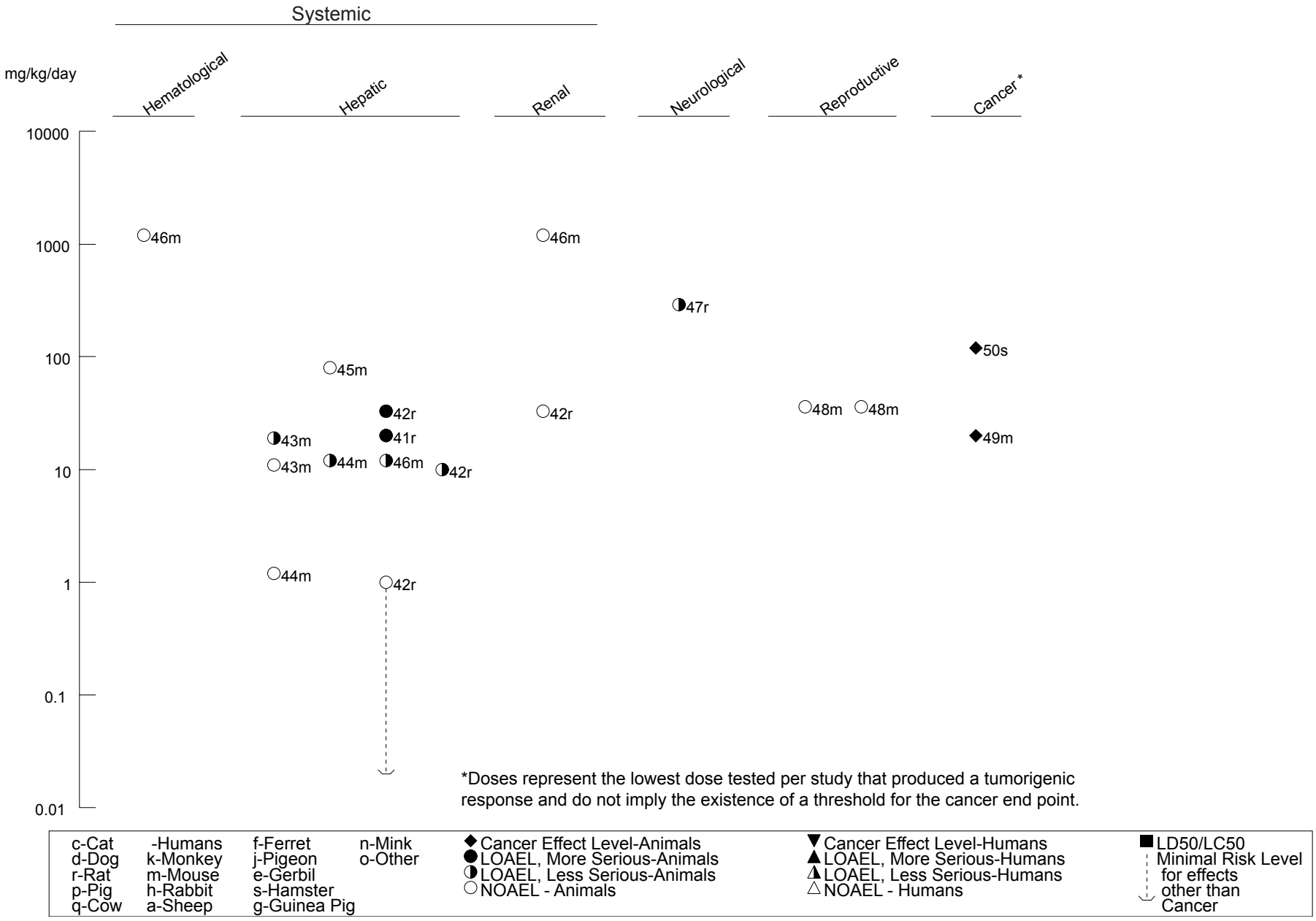

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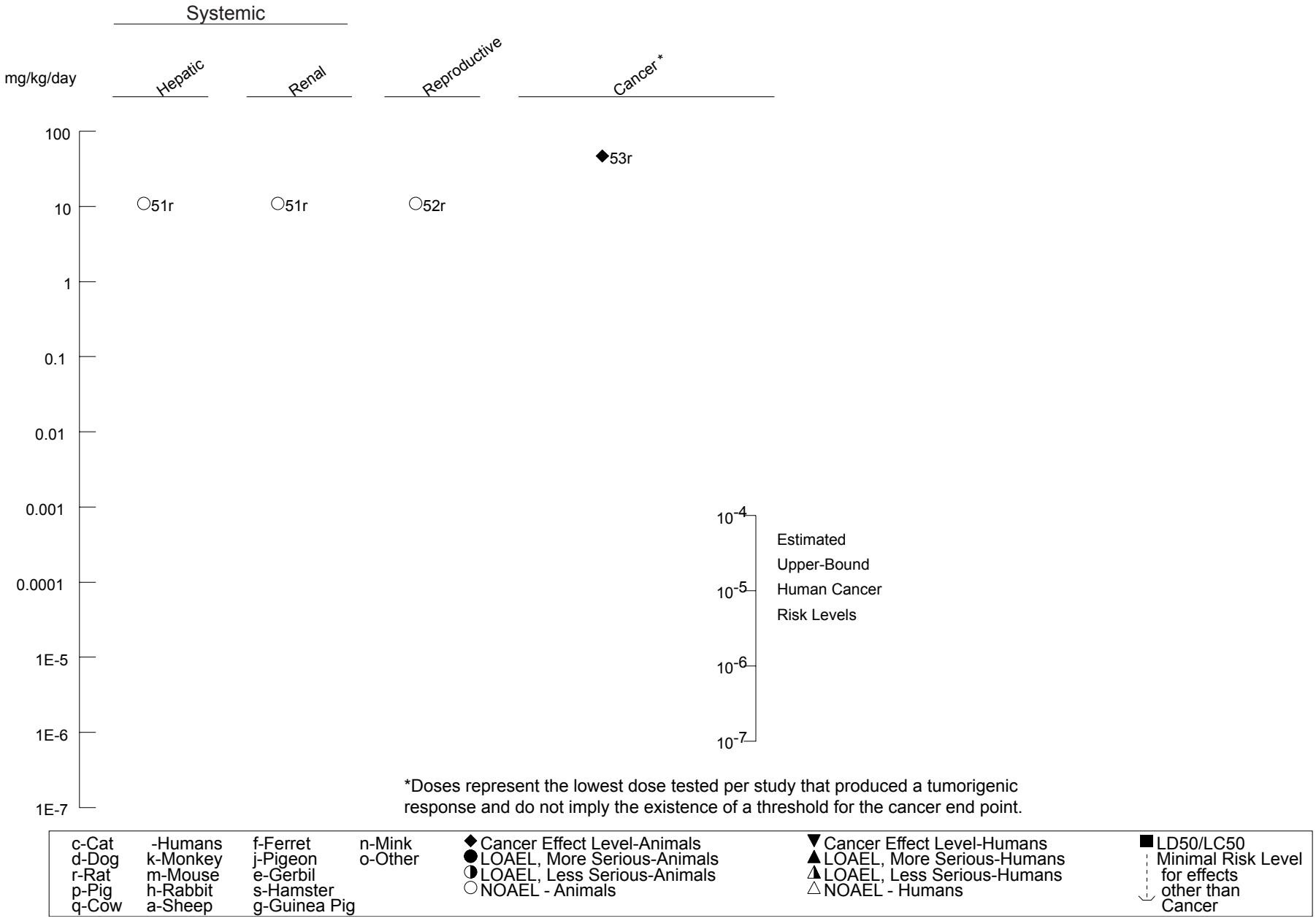
Figure 3-2. Levels of Significant Exposure to Carbon Tetrachloride - Oral (*Continued*)

Chronic (≥365 days)



discussed below. The highest NOAEL values and all LOAEL values for each reliable study for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Respiratory Effects. A number of human fatalities have been reported following ingestion of carbon tetrachloride (Umiker and Pearce 1953). Edema and hemorrhage of the lung were common autopsy findings. Injury to the lung usually did not become apparent until 8 days or longer after poisoning, and the effects on the lung were essentially the same as observed in cases of uremia due to other causes. This suggests that the late-developing edema and hemorrhagic injury to lung is secondary to severe kidney injury.

In animals, acute oral exposure to doses of 4,000 mg/kg has been observed to cause respiratory edema, atelectasis, and hemorrhage (Gould and Smuckler 1971). This is accompanied by marked disruption of subcellular structure in most pulmonary cell types, including granular pneumocytes, capillary endothelial cells, and Clara cells (Boyd et al. 1980; Gould and Smuckler 1971; Hollinger 1982). It has been shown that Clara cells were most severely injured because they are the most active in metabolic activation of carbon tetrachloride. Injury to capillary endothelial cells is dose-dependent, with increased release of cellular enzymes occurring at doses as low as 160 mg/kg (Hollinger 1982). No studies of respiratory effects following longer-term oral exposure were located.

Cardiovascular Effects. Effects of carbon tetrachloride ingestion on the cardiovascular system have not been the subject of extensive investigation. Most studies in humans have not detected significant gross or histopathological changes in heart tissue at dose levels that cause marked hepatic and renal damage (Leach 1922; MacMahon and Weiss 1929). Electrocardiographic changes (sinus arrhythmia, QRS complex splintering, elevated S-T_4 and P-R intervals) suggestive of myocardial injury were seen in a man who ingested several mouthfuls of carbon tetrachloride, but these appeared to be fully reversible (Conaway and Hoven 1946).

The few animal studies located appear to be in general agreement with the human findings (Gardner et al. 1925; Korsrud et al. 1972). Effects of carbon tetrachloride ingestion on blood pressure are sometimes observed, but these are likely secondary to effects on the central nervous system, or to effects on fluid and electrolyte balance following renal injury.

Gastrointestinal Effects. Humans who ingest oral doses in excess of 30 or 40 mL (680–910 mg/kg) frequently experience nausea, vomiting, and abdominal pain (Hardin 1954; New et al. 1962; Smetana

1939; Umiker and Pearce 1953; von Oettingen 1964). Nausea has been reported after an oral dose of as little as 100 mg/kg (Ruprah et al. 1985). These effects could be the direct result of irritation of the gastrointestinal tract caused by the high dose or secondary to effects on the central nervous system. Oral doses of 3–5 mL (70–110 mg/kg) were widely used in the past for the treatment of hookworms with only mild gastrointestinal distress (Hall 1921; Leach 1922).

No studies were located regarding gastrointestinal effects in animals after oral exposure to carbon tetrachloride.

Hematological Effects. Oral exposure to carbon tetrachloride has not been reported to have substantial direct hematological effects in humans or animals. Focal hemorrhagic lesions and mild anemia are sometimes observed in humans who have ingested carbon tetrachloride (Guild et al. 1958; Stewart et al. 1963), but this is likely due to decreased hepatic synthesis and/or secretion of clotting factors.

Only one study was identified that examined the hematological effects of carbon tetrachloride in animals. Intermediate oral exposure of mice to carbon tetrachloride did not result in any consistently significant hematological changes (Hayes et al. 1986).

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after oral exposure to carbon tetrachloride.

Only a single animal oral study was located that described effects on skeletal muscles. Male rats were exposed once per week by gavage to carbon tetrachloride doses of approximately 260–1,300 mg/kg/day, for either 3 or 10 weeks (Weber et al. 1992). Phenobarbital was also administered to hasten the onset of the typical signs of carbon tetrachloride-induced liver damage (inflammation, necrosis, fibrosis). Histological examination of various muscle tissues revealed no evidence of necrosis or inflammation, a finding supported by normal plasma levels of albumin, creatinine, creatinine phosphokinase, and urea nitrogen. However, muscle atrophy was observed that was apparently selective for fast glycolytic fibers, but not fast or slow oxidative fibers. This was shown to result from increased protein catabolism, and not from decreased protein synthesis. Although the mechanisms are not clearly understood, this muscle effect may be secondary to induced hepatic damage. This conclusion was partially inferred from the observed complete lack of myocyte necrosis, the fiber selectivity of the effect, the absence of enhanced catabolism in muscle exposed directly *in vitro* to 10-fold higher concentrations of carbon tetrachloride, the

elimination of disuse atrophy as a factor, and the correlation of this effect with only liver inflammation and necrosis, not cirrhosis (a condition which has been associated in humans with a negative nitrogen balance).

Hepatic Effects. Ingestion of carbon tetrachloride can lead to marked hepatotoxicity. In most reports involving humans, exposure has involved ingestion of several mouthfuls or more (probably 500 mg/kg or higher). Typical clinical signs of hepatic damage in such patients include a swollen liver, along with elevated serum levels of hepatic enzymes and decreased serum levels of liver-synthesized proteins (e.g., albumin, fibrinogen). In cases of death (usually occurring within 1–15 days), typical histological findings include fat accumulation, hepatic degeneration, and moderate to severe centrilobular necrosis; hepatitis was also diagnosed (Ashe and Sailer 1942; Jennings 1955; MacMahon and Weiss 1929; Umiker and Pearce 1953).

Single oral doses of 3–5 mL (70–110 mg/kg) were widely used in the past for treatment of hookworm, and ingestion of this dose resulted in clinical signs of liver injury in only a small number of cases (Hardin 1954; Lamson et al. 1928). Single doses of 4–8 mL (90–180 mg/kg) were found to result in fat accumulation in liver in several individuals (Docherty and Burgess 1922; Docherty and Nicholls 1923), and doses of only 1 mL (child) and 3 mL (adult) (approximately 80 mg/kg) have resulted in hepatic necrosis and death in a few cases (Phelps and Hu 1924). These results are indicative of differential susceptibility to carbon tetrachloride in humans. Certain confounding variables (age) may have been contributing factors to lethality at lower dose levels. One of the two cases involved a 5-year-old child, while the second report involved an adult; however, factors that may have increased susceptibility to the compound in this case could not be determined (Phelps and Hu 1924). No studies were located regarding the effects of longer-term or chronic oral exposure in humans to carbon tetrachloride.

The hepatotoxic effects of carbon tetrachloride have been widely studied in animals. Indeed, carbon tetrachloride is used as a model chemical in many laboratory investigations of the basic mechanism of action of hepatotoxic chemicals. Oral exposure to carbon tetrachloride has been observed to result in a wide spectrum of adverse effects on the liver, the most prominent of which are destruction of the smooth and rough endoplasmic reticulum and its associated enzyme activities (Reynolds and Yee 1968), inhibition of protein synthesis (Lutz and Shires 1978), impaired secretion of triglycerides with resultant fat accumulation (Fischer-Nielsen et al. 1991; Recknagel and Ghoshal 1966; Recknagel and Glende 1973; Waterfield et al. 1991), centrilobular necrosis (Blair et al. 1991; Reynolds and Yee 1968; Waterfield et al.

1991; Weber et al. 1992), and eventually fibrosis and cirrhosis (Allis et al. 1990; Bruckner et al. 1986; Fischer-Nielsen et al. 1991; Weber et al. 1992).

Although the occurrence of these effects has been confirmed in a very large number of studies, only a few investigations have focused on the dose-dependency of hepatic injury. After a single oral dose of 1,600 mg/kg to rats, urinary taurine levels were significantly increased ($p<0.01–0.05$) within 24 hours and liver weight was reduced (Waterfield et al. 1991). During the first 48 hours after a higher dose (3,200 mg/kg), first liver, then serum, and finally urinary levels of taurine were elevated. Similar effects, as well as reduced hepatic microsome levels of cytochrome P-450, aminopyrine demethylase, and aniline hydroxylase, were observed in rats after a single oral dose of 480 mg/kg/day (Thakore and Mehendale 1991). These effects were much more severe after 8,000 mg/kg, a dose found to be lethal within 14 days for most animals. Additionally, the liver evidenced necrosis, lipid vacuolation, and inflammation, and serum alanine and aspartate amino transferase levels were elevated. Single oral doses of only 40–80 mg/kg have also been observed to produce liver injury in rats and mice (Bruckner et al. 1986; Eschenbrenner and Miller 1946). When exposure was continued for 10–11 days, doses of 5–40 mg/kg/day produced mild signs of liver change, while 80 mg/kg/day caused clear hepatic injury (Bruckner et al. 1986; Smialowicz et al. 1991). The dose of 5 mg/kg/day from the latter study has been employed to calculate an acute oral MRL of 0.05 mg/kg/day, as described in the footnote on Table 3-2. At this dose, the earliest sign (vacuolar degeneration) of hepatocyte toxicity was just detectable. The severity of this hepatocellular injury with accompanying necrosis increased in a dose-related manner from 10 to 40 mg/kg/day.

In rats ingesting carbon tetrachloride for 12 weeks, no effects were detected at a dose of 1 mg/kg/day, mild centrilobular vacuolization was seen at 10 mg/kg/day, and extensive degenerative lesions were noted at 33 mg/kg/day (Bruckner et al. 1986). Results of other studies support these observations. Doses of 12–40 mg/kg/day produced mild signs of liver injury (as judged by fat accumulation, enzyme release or histological appearance) in mice and rats exposed for 35–90 days, while higher doses produced a dose-dependent increase in the extent and severity of liver injury (Alumot et al. 1976; Fischer-Nielsen et al. 1991; Hayes et al. 1986; Weber et al. 1992). Centrilobular hepatocellular vacuolar degeneration, necrosis, and cirrhosis were also found at dose levels of 20–40 mg/kg/day or greater for 12 weeks (Allis et al. 1990). All of these authors found the liver to be the organ most sensitive to carbon tetrachloride poisoning. Based on the NOAEL of 1 mg/kg/day in rats that was reported by Bruckner et al. (1986), an intermediate oral MRL of 0.02 mg/kg/day was calculated as described in the footnote in Table 3-2.

The hepatic effects of chronic oral exposure to carbon tetrachloride have not been well studied. Alumot et al. (1976) reported no significant effects on serum enzyme levels or hepatic fat content of rats exposed to doses of approximately 11–14 mg/kg/day for 2 years. It should be noted that this dose level is somewhat higher than that found to cause hepatic effects following intermediate exposure (Bruckner et al. 1986). The most important factor in the difference between the results of the studies of Alumot et al. (1976) and Bruckner et al. (1986) is most likely the difference in dosage regimen. Bruckner et al. (1986) gave the daily dose of carbon tetrachloride by bolus gavage, while Alumot et al. (1976) administered carbon tetrachloride in the rats' diet. Bruckner et al. (1990) clearly demonstrated that a single oral bolus dose produces much higher blood levels of carbon tetrachloride and greater hepatic injury than does the same amount of carbon tetrachloride given in divided doses over a period of hours (as would occur upon ingestion of carbon tetrachloride in the diet). Variations might also be due to other experimental protocol differences (e.g., strain variations, differences in end points monitored), or it might be development of resistance upon repeated exposure resulting from carbon tetrachloride-induced destruction of the liver cytochrome P-450 enzyme system that is required for its own metabolic activation. No chronic oral MRL was derived because of a lack of suitable dose-response data.

Renal Effects. Nephritis is a common finding in fatal cases of carbon tetrachloride ingestion in humans (Umiker and Pearce 1953), and renal failure may contribute to death in many cases (Gosselin et al. 1976; von Oettingen 1964). Typically, clinical signs of renal dysfunction (oliguria or anuria, albuminuria, proteinuria, elevated blood urea nitrogen edema, hypertension) tend to develop within 1–6 days after exposure, somewhat later than the appearance of hepatic injury (Conaway and Hoven 1946; Guild et al. 1958; Kluwe 1981; MacMahon and Weiss 1929; Smetana 1939; Umiker and Pearce 1953). In nonfatal cases, renal function usually returns to normal within several weeks (Guild et al. 1958; Kluwe 1981; Smetana 1939). Histological changes in the kidney are observed primarily in the proximal tubular epithelium, where cells become swollen and granular, with moderate to severe necrosis (Docherty and Burgess 1922; Guild et al. 1958; MacMahon and Weiss 1929; Smetana 1939).

Studies in animals confirm that the kidney is a target tissue for carbon tetrachloride, although in rodents, the kidney is much less sensitive than the liver to carbon tetrachloride. Doses of 4,000 mg/kg resulted in swollen and pale kidneys in rats within 2 days, with morphological changes present primarily in proximal tubular epithelial cells. All histological and functional signs of renal injury were fully reversible within 5 days (Striker et al. 1968). Fatty degeneration of the kidney has been observed in dogs after a single dose of 3,200 mg/kg (Chandler and Chopra 1926) and swelling of the convoluted tubules after 6,400 mg/kg (Gardner et al. 1925). Exposure of rats to 160 mg/kg/day for about 10 days did not induce

adverse renal effects (Bruckner et al. 1986; Smialowicz et al. 1991), nor did 12 weeks exposure to 33 mg/kg/day, 5 days/week (Bruckner et al. 1986). Only marginal indication of kidney injury was detected in mice exposed to doses of 2,500 mg/kg/day for 14 days or 1,200 mg/kg/day for 90 days (Hayes et al. 1986). It should be recalled that these doses result in marked hepatotoxicity.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after oral exposure to carbon tetrachloride.

Studies in rodents have shown significant suppression of immune function following exposure to carbon tetrachloride. Exposure of female mice to carbon tetrachloride at 500 mg/kg/day for 7 consecutive days suppressed the T-cell-dependent humoral responses to sheep red blood cells (SRBC) (Delaney et al. 1994). The effect was mediated by an increase in serum levels of transforming growth factor beta-1 (TGF-beta-1), which occurred 24–48 hours after exposure in single-dose experiments (at 250–500 mg/kg, but not 50 mg/kg). Exposure of rats to carbon tetrachloride (up to 160 mg/kg/day for 10 days) by gavage did not alter the primary antibody response to SRBC, lymphoproliferative responses to mitogen or mixed leukocytes, natural killer cell activity, or cytotoxic T-lymphocyte responses; also, spleen and thymus weights were comparable to controls (Smialowicz et al. 1991). In female mice that were given daily gavage doses between 50 and 500 mg/kg/day for 14 days (sufficient for hepatotoxicity), the T-cell-dependent humoral response to SRBC was suppressed at ≥50 mg/kg/day, serum anti-SRBC IgM titers were reduced at 100 mg/kg/day, and the absolute number and percentage of $CD4^{+}CD8^{-}$ T-cells per spleen was reduced at 500 mg/kg/day (Guo et al. 2000). Exposure had no effect on the mixed leukocyte response to allogenic spleen cells, or the activities of cytotoxic T-lymphocytes or natural killer (NK) cells. In this study, exposure to carbon tetrachloride also decreased host resistance to *Streptococcus pneumoniae* and *Listeria monocytogenes*, with the effective dose dependent on the magnitude of the challenge. In rats exposed twice weekly for 4–12 weeks to 3,688 mg/kg/day, there was histologic evidence of hemorrhage, hemosiderin deposition, and lymphocyte depletion in the pancreaticoduodenal lymph node (Doi et al. 1991), an effect that may be secondary to induced hepatic damage.

The highest NOAEL values and all LOAEL values for each reliable study of immunological and lymphoreticular effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

Ingestion of carbon tetrachloride frequently results in marked depression of the central nervous system. Neurological signs in humans include headache, vertigo, weakness, blurred vision, lethargy, and coma, sometimes accompanied by tremor and parasthesias. Mental confusion and disorientation tend to appear later. These symptoms have been reported in people who ingested single oral doses of carbon tetrachloride ranging from 5 to 473 mL (approximately 114–10,800 mg/kg) (Cohen 1957; Leach 1922; Stevens and Forster 1953; Stewart et al. 1963). The onset of initial effects is very rapid, and is likely the result of direct narcotic action on the central nervous system, similar to other anesthetic halocarbons. Recovery from the depressant effects generally appears to be complete (Stevens and Forster 1953; Stewart et al. 1963), although in some fatal cases, histological examination of the brain has revealed patchy pontine necrosis, demyelination, and Purkinje cell damage, with widespread hemorrhagic infarcts (Cohen 1957). Single oral doses of 70 or 120 mg/kg have been reported to be without significant neurological effect (Hall 1921; Leach 1922).

Only one animal study was located that specifically reported neurological effects other than those that typically attend acute high-dose exposure (e.g., lethargy, coma, related cardiac effects of arrhythmia, and blood pressure changes). When rats pretreated with phenobarbitol received weekly doses of carbon tetrachloride for 10 weeks (initially 289 mg/kg/day, increasing to a maximum of approximately 1,600 mg/kg/day according to body weight gain), a condition of diffuse micronodular liver cirrhosis was induced (Bengtsson et al. 1987). This was accompanied by significantly increased synthesis of the neurotransmitter serotonin in all six areas of the brain that were monitored. Serotonin levels were not, however, reliably correlated with any abnormal open-field behavior, which was used as an indicator of the possible portal-systemic encephalopathy that may accompany liver failure.

The highest NOAEL values and all LOAEL values for each reliable study for neurological effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to carbon tetrachloride.

Rats (males and females) ingested carbon tetrachloride in their food for 5–6 weeks (Alumot et al. 1976). No effects were noted on most reproductive parameters monitored (percent conception, percent with litters, mean litter size, mean body weight of offspring at birth and at weaning). An increase in neonatal mortality was observed in the low dose group (about 6 mg/kg/day), but not in the high dose group (about 15 mg/kg/day). The authors concluded that this response was not treatment related, and that these doses of carbon tetrachloride had no adverse effect on reproduction.

The highest NOAEL values for reproductive effects in rats after chronic exposure are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

An epidemiological study was conducted using birth outcome and drinking water exposure databases from a four-county area in northern New Jersey (Bove et al. 1992a, 1992b, 1995). The cross-sectional study of data from 75 out of 146 towns spanned the period 1985–1988 and evaluated the entire study population of 80,938 singleton live births and 599 singleton fetal deaths. Initial conclusions were based solely on state and institutional records, with no interviews conducted (Bove et al. 1992a). Estimated carbon tetrachloride concentrations in the drinking water of >1 ppb were associated with the following adverse developmental outcomes (odds ratio, 95% confidence interval, significance): full-term birth weight <2,500 g (2.26, 1.41–3.6, $p<0.001$), small for gestational age (1.35, 1.03–1.8, $p<0.03$), central nervous system defects (4.64, 0.93–14.2, $p<0.065$), neural tube defects (5.39, 1.31–22.2, $p<0.025$), and cleft-lip or cleft-palate (3.60, 0.88–14.7, $p<0.08$). Although sacrificing substantial statistical power and risking the introduction of certain sampling biases, a case control (43–49 cases per outcome, 138 controls) examination with case-mother interviews was also conducted in an attempt to better account for possible confounding risk factors (Bove et al. 1992b). However, adjustment by risk factor variables from the interviews had no significant effect on the unadjusted results for carbon tetrachloride. Methodological limitations of the study may have resulted in chance, missed, or under- or overestimated associations. As acknowledged by the authors, inhalation and/or dermal exposure through bathing and showering could be at least as significant as the oral exposure. Although these studies suggest a causative role for carbon tetrachloride in the generation of certain adverse developmental outcomes, issues that could beneficially be addressed in the future include better-defined exposure levels (these levels appear to be rather low for a causative agent) and the potential for such effects to be the result of complex mixture exposure.

A case-control study of selected congenital malformations and maternal residential proximity to NPL sites in California between 1989 and 1991 did not find an increased risk of conotruncal heart defects or oral cleft defects associated with sites containing carbon tetrachloride (Croen et al. 1997).

No teratogenic effects were reported in rats following maternal oral exposure to carbon tetrachloride, but total resorption of fetuses was reported at maternally toxic doses. Doses of 1,400 mg/kg/day during gestation caused marked maternal toxicity in rats, and total resorption of fetuses in some animals, but no adverse effects in surviving litters (Wilson 1954). In rats treated with carbon tetrachloride by gavage in corn oil or an aqueous vehicle (Emulphor EL-620) on gestational days 6–15, no maternal or developmental toxicity occurred at a dose of 25 mg/kg/day (Narotsky et al. 1997a). Total loss of some litters and clinical signs of toxicity (piloerection and reduced body weight gain) occurred in dams treated with ≥50 mg/kg/day. Effects were slightly more severe when the vehicle was corn oil (5/12 litters resorbed) than when an aqueous vehicle was used (2/24 litters resorbed). Dams treated with 75 mg/kg/day in corn oil exhibited body weight losses during gestational days 6–8.

Temporal variations during gestation in sensitivity to carbon tetrachloride were reported in rats. When pregnant rats were given a single dose of 150 mg/kg carbon tetrachloride on gestational day 6, 7, 8, 10, or 12, the incidences of full litter loss ranged between 36 and 72% during gestation days 6–10 (maximal day 8) and 0% on day 12 compared to 4% for the controls (Narotsky et al. 1997b). The authors concluded that gestational days 6–10 represented a critical period of vulnerability to carbon tetrachloride in rats. Dams later found to have had full litter resorption exhibited bloody vaginal discharges within 24 hours of dosing. No additional developmental toxicity was reported in surviving litters. Offspring were not evaluated for possible neurobehavioral deficits.

The highest NOAEL value for developmental effects in rats after acute exposure is recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

No studies were located regarding carcinogenic effects in humans after oral exposure to carbon tetrachloride.

Studies in animals (rats, hamsters, and several strains of mice) provide convincing evidence that ingestion of carbon tetrachloride increases the risk of liver cancer (Andervont 1958; Della Porta et al. 1961;

Edwards 1941; Edwards et al. 1942; Eschenbrenner and Miller 1944, 1946; NCI 1976). In general, carbon tetrachloride-induced liver tumors were either hepatomas or hepatocellular carcinomas that appeared after exposure periods of only 10–30 weeks (Edwards 1941; Eschenbrenner and Miller 1944; NCI 1976). For example, daily oral doses as low as 20 mg/kg produced hepatic tumors in mice exposed for 120 days (Eschenbrenner and Miller 1946). In most cases, the incidence of hepatic tumors was very high (75–100%) in exposed animals. In each of these studies, the carbon tetrachloride was administered by single bolus gavage. As noted in the discussion of oral hepatic effects, such a dosing regimen may exacerbate cancer effects relative to those that might be observed under conditions of food or drinking water exposure. Based on these studies, both IARC (1987) and EPA (IRIS 1993) have concluded there is sufficient evidence that carbon tetrachloride is carcinogenic in experimental animals, and that it is possibly or probably carcinogenic in humans.

The EPA (1984) reviewed the available information on the carcinogenic effects of carbon tetrachloride following oral exposure, and concluded that the studies by Della Porta et al. (1961) in hamsters, Edwards et al. (1942) in mice, and NCI (1976) in rats and mice had adequate dose-response data to allow quantitative estimation of the unit cancer risk (the excess risk of cancer associated with lifetime ingestion of water containing 1 μg/L, assuming intake of 2 L/day by a 70-kg person). Since each study was judged to have some limitations, no one study was selected as the basis for the risk calculation. Rather, calculations were performed for all four data sets, and the geometric mean of these estimates was taken to be the most appropriate value. These calculations are summarized in Table 3-3. Because of the uncertainty in the data and in the calculations, the EPA identified the geometric mean of the upper 95% confidence limit ($3.7x10^{-6}$) as the preferred estimate of unit cancer risk.

Based on this value, the upper-bound lifetime risk from ingestion of 1 μg/kg/day of carbon tetrachloride is $1.3x10^{-4}$, and the daily intake levels associated with lifetime risks of 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} are 0.77, 0.077, 0.0077, and 0.00077 μg/kg/day, respectively.

Because these are based on upper-bound estimates, the true risk could be lower. These values, along with doses of carbon tetrachloride that have been observed to cause cancer in animals, are presented in Figure 3-2.

Table 3-3. Summary of Carcinogenic Unit Risk Calculations for Oral Exposure to Carbon Tetrachloride[a]

		Unit cancer risk[b]	
Reference	Species	Best estimate	Upper 95% confidence limit
Della Porta et al. (1961)	Hamster	2.5×10^{-5}	3.4×10^{-5}
Edwards et al. (1942)	Mouse	7.1×10^{-6}	9.4×10^{-6}
NCI (1976)	Mouse	1.4×10^{-6}	1.8×10^{-6}
NCI (1976)	Rat	1.9×10^{-7}	3.1×10^{-7}
	Geometric Mean	2.5×10^{-6}	3.7×10^{-6}

[a]Source: EPA 1984
[b]The estimated probability of cancer in a 70-kg person ingesting 2 L/day of water containing 1 µg/L of carbon tetrachloride for a lifetime

3.2.3 Dermal Exposure

3.2.3.1 Death

A number of cases of fatal or near-fatal exposure to carbon tetrachloride have been reported following its use as a dry shampoo or as a solvent for removal of adhesives from skin (Chandler 1936; Hardin 1954). However, these cases almost certainly involved inhalation exposure as well as dermal exposure, and no quantitative estimate of a lethal dermal dose in humans was located.

In animals, a dose of 260 mg/cm^2 applied to the occluded skin of guinea pigs resulted in 25% mortality within 5 days, with 65% mortality at a dose of 1,000 mg/cm^2 (Wahlberg and Boman 1979). The dermal LD_{50} was estimated to be greater than 15,000 mg/kg in rabbits and guinea pigs that were exposed to carbon tetrachloride (occluded) for 24 hours (Roudabush et al. 1965).

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, hematological, musculoskeletal, or ocular effects after dermal exposure of humans or animals to carbon tetrachloride. Gastrointestinal, hepatic, renal, and dermal effects were reported in humans. Hepatic and dermal effects were also seen in animals. These effects are discussed below. The LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-4.

Gastrointestinal Effects. There are case reports of three humans who experienced gastrointestinal symptoms, including nausea and vomiting, after dermal application of carbon tetrachloride-based lotion (Perez et al. 1987). No quantitative estimate of the amount of carbon tetrachloride applied or absorbed was provided.

No studies were located regarding gastrointestinal effects in animals after dermal exposure to carbon tetrachloride.

Hepatic Effects. Liver injury, characterized by an elevated serum enzyme (alanine aminotransferase level), was described in case reports of three humans after dermal application of carbon tetrachloride (Perez et al. 1987). In the absence of quantitative estimates of the amount of carbon tetrachloride applied or absorbed, NOAEL and LOAEL values cannot be determined.

Table 3-4 Levels of Significant Exposure to Carbon Tetrachloride - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL				Reference Chemical Form
				Less Serious		Serious		
ACUTE EXPOSURE								
Death								
Gn Pig	Once 24 hr					15000 mg/kg	(LD50, 24 hours)	Roudabush et al. 1965
Gn Pig	Once contact for 5 d					260 mg/cm²	(5/20)	Wahlberg and Boman 1979
Rabbit	Once 24 hr					15000 mg/kg	(LD50, 24 hours)	Roudabush et al. 1965
Systemic								
Gn Pig	Once 15 min-16 hr	Hepatic		513 mg/cm²	(hydropic changes, slight necrosis)			Kronevi et al. 1979
		Dermal		513 mg/cm²	(karyopynosis, spongiosis, perinuclear edema)			
Gn Pig	Once 24 hr	Dermal		120 mg/kg/day	(primary irritation)			Roudabush et al. 1965
Rabbit	Once 24 hr	Dermal		120 mg/kg/day	(primary irritation)			Roudabush et al. 1965

cm = centimeters; d = day(s); Derm = dermal; Gn pig = Guinea pig; hr = hour(s); LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; mg/kg/day = milligrams per kilograms per day; NOAEL = no-observed-adverse-effect level

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Hydropic changes and isolated necrotic areas were reported in the liver of guinea pigs 16 hours after dermal contact with 513 mg/cm^2 of carbon tetrachloride (Kronevi et al. 1979). An area of 3.1 cm^2 of clipped skin was encompassed by gluing a glass ring to the animals' backs. After administering 1.0 mL of the substance, the ring was covered by attaching a cover glass.

Renal Effects. Acute renal failure, as evident by anuria and azoturia, was reported in three case reports of humans after dermal application of carbon tetrachloride-based lotion (Perez et al. 1987). The usefulness of this finding is limited by the lack of data concerning the amount of carbon tetrachloride applied or absorbed.

No studies were located regarding renal effects in animals after dermal exposure to carbon tetrachloride.

Dermal Effects. In humans, direct dermal contact with undiluted carbon tetrachloride causes a mild burning sensation with mild erythema (Stewart and Dodd 1964). Some individuals appear to be hypersensitive, developing marked swelling, itching, and blisters following dermal contact (Taylor 1925).

Similar effects of dermal contact with carbon tetrachloride have been described in animals. A dose of 124 mg/cm^2 carbon tetrachloride produced moderate primary irritation within 24 hours when applied occluded to the intact or abraded skin of rabbits or guinea pigs, with irritation scores of 2.2–4.1 on skin (Roudabush et al. 1965). Direct dermal contact of guinea pigs with liquid carbon tetrachloride (occluded; 513 mg/cm^2) caused degenerative changes in epidermal cells and marked intercellular edema or spongiosis (Kronevi et al. 1979). These effects became apparent within 15 minutes, and progressed in severity over the course of several hours. These effects require direct dermal contact because similar effects on the skin are not observed following inhalation or oral exposure.

3.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans or animals after dermal exposure to carbon tetrachloride.

3.2.3.4 Neurological Effects

A case of polyneuritis was reported in a man who had repeated dermal contact 8 hours/day with carbon tetrachloride using it as a degreasing agent (Farrell and Senseman 1944).

No studies were located regarding neurological effects in animals after dermal exposure to carbon tetrachloride.

3.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after dermal exposure to carbon tetrachloride.

3.2.3.6 Developmental Effects

No studies were located exclusively regarding developmental effects in humans or animals after dermal exposure to carbon tetrachloride. However, note the epidemiological studies discussed in Section 3.2.2.6, which almost certainly involved significant dermal and inhalation exposures in addition to the emphasized oral exposure.

3.2.3.7 Cancer

No studies were located regarding carcinogenic effects in humans or animals following dermal exposure to carbon tetrachloride.

3.3 GENOTOXICITY

The genotoxic potential of carbon tetrachloride has been evaluated *in vivo* (oral and intraperitoneal injection exposures) and *in vitro*.

Inhalation Exposure. No studies were located on genetic effects in humans or animals after inhalation exposure to carbon tetrachloride.

Oral Exposure. No studies were located regarding genetic effects in humans after oral exposure to carbon tetrachloride.

Oral exposure of rats to a single dose of 40–400 mg/kg carbon tetrachloride did not result in unscheduled DNA synthesis in hepatocytes isolated from the treated animals (Mirsalis and Butterworth 1980; Mirsalis et al. 1982). In a similar experiment, Craddock and Henderson (1978) found that oral exposure of rats to carbon tetrachloride caused an increase in DNA synthesis associated with tissue regeneration, but no increase in unscheduled DNA synthesis. Furthermore, chromosome aberrations, micronuclei, or sister chromatid exchanges were not induced within 4–72 hours in hepatocytes taken from rats treated with the relatively high oral dose of 1,600 mg/kg (Sawada et al. 1991). No increase in the frequency of micronuclei was detected in mouse bone marrow following gavage doses as high as 2,000 mg/kg (Suzuki et al. 1997). DNA damage was detected electrophoretically (comet assay) in the livers of male CD-1 mice 24 hours after administration of gavage doses of 1,000 or 2,000 mg/kg (Sasaki et al. 1998); no increase was observed in the liver at 500 mg/kg and results were negative at 500–2,000 mg/kg for other tissues (stomach, kidney, bladder, lung, brain and bone marrow).

Some genotoxicity of carbon tetrachloride is related to the lipid peroxidation activity of its metabolites. Four days after administration of carbon tetrachloride to Sprague-Dawley rats (sex unspecified) as a single oral dose of 0.1 mg/kg, the levels of the lipid peroxidation products isoprostane and malondialdehyde were elevated 16- and 3.5-fold, respectively, over background in the livers (Chaudhary et al. 1994). In these animals, the level of the adduct malondialdehyde deoxyguanosine in hepatic DNA was elevated 1.8-fold over background levels. These studies are summarized in Table 3-5.

Dermal Exposure. No studies were located regarding genotoxic effects in humans or animals after dermal exposure to carbon tetrachloride.

Other Routes of Exposure. Some metabolism-dependent genotoxicity of carbon tetrachloride may be the result of the lipid peroxidation activity of its metabolites (Chung et al. 2001; Wacker et al. 2001). *Trans*-4-hydroxy-2-nonenal is a genotoxic product of lipid peroxidation that occurs at low background levels in rodent tissues. A single intraperitoneal injection of 500 mg/kg carbon tetrachloride into female F344 rats resulted in significant increases in the levels of 1,N^2-propanodeoxyguanosine, a deoxyguanosine adduct of *trans*-4-hydroxy-2-nonenal, at high levels in the forestomach and liver, and to a lesser degree in the lung, colon, and kidney (Wacker et al. 2001). Increases were in the order of 1.5–2-fold higher than background

Table 3-5. Genotoxicity of Carbon Tetrachloride *In Vivo*

Species (test system)	End point	Results	Reference
Oral route:			
Rat hepatocytes	Chromosomal aberrations	–	Sawada et al. 1991
Rat hepatocytes	Sister chromatid exchange	–	Sawada et al. 1991
Rat hepatocytes	Micronuclei	–	Sawada et al. 1991
Rat hepatocytes	Unscheduled DNA synthesis	–	Mirsalis and Butterworth 1980
Rat hepatocytes	Unscheduled DNA synthesis	–	Mirsalis et al. 1982
Rat hepatocytes	Unscheduled DNA synthesis	–	Craddock and Henderson 1978
Rat liver	DNA adducts (lipid peroxidation)	+	Chaudhary et al. 1994
Mouse liver, stomach, kidney, bladder, lung, brain, bone marrow	DNA damage (comet assay) after 3 hours	–	Sasaki et al. 1998
Mouse stomach, kidney, bladder, lung, brain, bone marrow	DNA damage (comet assay) after 24 hours	–	Sasaki et al. 1998
Mouse liver	DNA damage (comet assay) after 24 hours	+	Sasaki et al. 1998
Mouse bone marrow	Micronuclei	–	Suzuki et al. 1997
Intraperitoneal injection:			
Rat forestomach, liver, lung, colon, kidney	DNA adducts (lipid peroxidation)	+	Wacker et al. 2001
Rat liver	DNA adducts (lipid peroxidation)	+	Chung et al. 2001
Mouse peripheral lymphocytes	Micronuclei	–	Suzuki et al. 1997

– = negative result; + = positive result; DNA = deoxyribonucleic acid

in the 4–24 hours following treatment. One intraperitoneal injection at a dose of 3,200 mg/kg into male F344 rats resulted in a ~37-fold increase over background of 1,N^2-propanodeoxyguanosine levels in the liver (Chung et al. 2001). No increase in micronucleus formation was detected in mouse peripheral blood reticulocytes 24–72 hours following intraperitoneal injection doses as high as 3,000 mg/kg (Suzuki et al. 1997).

These studies are summarized in Table 3-5.

In Vitro. Most *in vitro* studies of the mutagenic potential of carbon tetrachloride have been negative, both in prokaryotic systems (Barber et al. 1981; Brams et al. 1987; Hellmer and Bolcsfoldi 1992; McCann et al. 1975; Simmon et al. 1977; Uehleke et al. 1977) and eukaryotic systems (Dean and Hodson-Walker 1979; Garry et al. 1990; Loveday et al. 1990).

Suggestive evidence for the genotoxicity of carbon tetrachloride was noted in several studies in yeast *(Saccharomyces cerevisiae)*. Increases in recombinants and revertants were observed at concentrations of carbon tetrachloride (34 mM) considerably above the solubility of carbon tetrachloride in water (5 mM) (Callen et al. 1980). In the RS112 diploid strain designed to detect intrachromosomal recombination, 4–8 mg/mL carbon tetrachloride yielded positive results, which was attributed to the observed increase in oxidative free radicals (Brennan and Schiestl 1998). The chemical induced intrachromosomal recombination in dividing cells or cells arrested in G1 or G2 phase, but not cells in S phase (Galli and Schiestl 1998). Evidence that the chemical prematurely pushed G1 cells into S-phase suggested that genotoxicity might result from the failure to completely repair DNA before replication, resulting in DNA strand breaks.

Carbon tetrachloride (0.01–1 mM) failed to induce unscheduled DNA synthesis in hepatocytes isolated from male CD rats (Selden et al. 1994). Treatment with 2–16 μg/mL carbon tetrachloride did not increase the frequency of chromosomal aberrations in peripheral lymphocytes isolated from Merino lambs, but the frequency of micronucleus formation was significantly increased at 8–16 μg/mL without activation and at 16 μg/mL with activation (Sivikova et al. 2001).

There is evidence that the biotransformation of carbon tetrachloride may produce DNA adducts directly or indirectly, as by-products of lipid peroxidation (Beddowes et al. 2003; Castro et al. 1997). In hepatocytes isolated from female Wistar rats, 1–4 mM carbon tetrachloride induced a small, statistically significant elevation in malondialdehyde deoxyguanosine adducts (the result of lipid peroxidation) and

DNA strand breaks (Beddowes et al. 2003). Increases in 8-oxodeoxyguanosine adducts were observed at the threshold of, and concomitant with, cytotoxicity. A biochemical study using DNA bases and liver microsomes from male Sprague-Dawley rats demonstrated that the bioactivation of carbon tetrachloride resulted in the formation of adducts to guanine (2,6-diamino-4-hydroxy-5-formamidopyrimidine), cytosine (5-hydroxycytosine), and thymidine (5-hydroxymethyluracil), but not to adenine (Castro et al. 1997). Adduct formation was attributed to reactive metabolites (trichloromethyl or trichloromethyl-peroxyl free radicals) or to reactive aldehydes, such as malondialdehyde, which are generated by lipid peroxidation.

These *in vitro* studies are summarized in Table 3-6.

3.4 TOXICOKINETICS

Carbon tetrachloride is absorbed readily from the gastrointestinal and respiratory tracts, and more slowly through the skin. It is distributed to all major organs, with highest concentrations in the fat, liver, bone marrow, adrenals, blood, brain, spinal cord, and kidney (Bergman 1983; Dambrauskas and Cornish 1970; McCollister et al. 1951; Paustenbach et al. 1986a, 1986b). Once carbon tetrachloride is absorbed, it is metabolized by cytochrome P-450 enzymes, with the production of the trichloromethyl radical (Lai et al. 1979; Poyer et al. 1978). Aerobically, metabolism of the trichloromethyl radical can eventually form phosgene (Shah et al. 1979). Anaerobically, the radical can undergo reactions to form chloroform (Glende et al. 1976; Uehleke et al. 1973), hexachloroethane (Fowler 1969; Uehleke et al. 1973), or carbon monoxide (Wolf et al. 1977), as well as bind directly to lipids, proteins, and deoxyribonucleic acid (DNA) (Rao and Recknagel 1969). Carbon tetrachloride is excreted primarily in exhaled air (initial elimination half-life of 1–3 hours) and in the feces, while relatively minimal amounts are excreted in the urine (McCollister et al. 1951; Paustenbach et al. 1986a; Stewart and Dodd 1964; Stewart et al. 1961, 1963, 1965; Young and Mehendale 1989).

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Although there are many cases of human overexposure to carbon tetrachloride vapor, there are few quantitative studies of pulmonary absorption of carbon tetrachloride in humans. Based on the difference in carbon tetrachloride concentration in inhaled and exhaled air, absorption across the lung was estimated

Table 3-6. Genotoxicity of Carbon Tetrachloride *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Escherichia coli (K-12 343/113)	Differential DNA repair	–	–	Hellmer and Bolcsfoldi 1992
E. coli PQ37	SOS induction (DNA repair)	–	–	Brams et al. 1987
Salmonella typhimurium (TA1535)	Reversion frequency	–	–	McCann et al. 1975
S. typhimurium (TA1535, TA1538)	Reversion frequency	–	No data	Uehleke et al. 1977
S. typhimurium	Reversion frequency	No data	–	Simmon et al. 1977
S. typhimurium (TA1535, TA98, TA100)	Reversion frequency	–	–	Barber et al. 1981
Eukaryotic organisms:				
Saccharomyces cerevisiae (D7)	Frequency of convertants recombinants, revertants	No data	+	Callen et al. 1980
S. cerevisiae (RS112)	DEL (intrachromosomal recombination)	NT	+	Brennan and Schiestl 1998
S. cerevisiae (RS112)	DEL (intrachromosomal recombination)	NT	+/– (see text)	Galli and Schiestl 1998
Mammalian cells:				
Rat liver cell line (RL_1)	Chromatid gaps, deletions or aberrations	No data	–	Dean and Hodson-Walker 1979
Rat hepatocytes (Wistar)	DNA strand breaks, adducts	NT	+	Beddowes et al. 2003
Rat hepatocytes (CD)	Unscheduled DNA synthesis	NT	–	Selden et al. 1994
Human/peripheral lymphocytes	Sister chromatid exchange	–	–	Garry et al. 1990
Human/peripheral lymphocytes	Chromosomal aberration	–	–	Garry et al. 1990
Lamb *(Ovis aries)*/peripheral lymphocytes	Chromosomal aberration	NT	–	Sivikova et al. 2001
Lamb *(Ovis aries)*/peripheral lymphocytes	Micronucleus formation	+	+	Sivikova et al. 2001
Chinese hamster ovary cells	Sister chromatid exchange	–	–	Loveday et al. 1990
Chinese hamster ovary cells	Chromosomal aberration	–	–	Loveday et al. 1990

[a]Not reported, but derived as a wide-dose range

– = negative result; + = positive result; NT = not tested

to be about 60% in humans (Lehmann and Schmidt-Kehl 1936). Monkeys exposed to 50 ppm absorbed an average of 30.4% of the total amount of carbon tetrachloride inhaled, at an average absorption rate of 0.022 mg carbon tetrachloride/kg/minute (McCollister et al. 1951). The concentration of carbon tetrachloride in the blood increased steadily, but did not reach a steady-state within 344 minutes of exposure. In rats exposed to 100 or 1,000 ppm for 2 hours, the total absorbed dose of carbon tetrachloride was 17.5 or 179 mg/kg of body weight, respectively (Sanzgiri et al. 1995). (These results were used to establish dose levels for parallel oral-route studies described in Section 3.4.1.2.) Carbon tetrachloride was rapidly absorbed from the lungs as indicated by the near peak levels that were measured in arterial blood at the earliest timepoint (5 minutes). A near steady-state was achieved within 10 or 15 minutes and was maintained for the duration of the 2-hour exposures. In rats, mice, and hamsters exposed to 20 ppm ^{14}C-labeled carbon tetrachloride vapor for 4 hours, the initial body burdens of carbon tetrachloride equivalents (CE) immediately following exposure were 12.1, 1.97, and 3.65 μmol, respectively (Benson et al. 2001).

3.4.1.2 Oral Exposure

No studies were located regarding absorption in humans after oral exposure to carbon tetrachloride. It would be anticipated, however, that carbon tetrachloride is well absorbed from the gastrointestinal tract of humans, since carbon tetrachloride is readily absorbed from the gastrointestinal tract of animals (see below), and there are many accounts of human poisonings resulting from ingestion of carbon tetrachloride (e.g., Ashe and Sailer 1942; Conway and Hoven 1946; Gosselin et al. 1976; Guild et al. 1958; Kluwe 1981; Lamson et al. 1928; Phelps and Hu 1924; Ruprah et al. 1985; Stewart et al. 1963; Umiker and Pearce 1953; von Oettingen 1964).

Results from several animal studies indicate that carbon tetrachloride is rapidly and extensively absorbed from the gastrointestinal tract. Typically, 80–85% of an oral dose may be recovered in expired air, indicating that gastrointestinal absorption is at least 85% (Marchand et al. 1970; Paul and Rubinstein 1963). The time course of absorption depends on exposure conditions, with peak blood levels occurring as early as 3–6 minutes after dosing (Kim et al. 1990a). While oral absorption from water or other aqueous vehicles is very rapid and extensive, when carbon tetrachloride is administered using corn oil as the vehicle, absorption is slowed and diminished (Gillespie et al. 1990; Kim et al. 1990a). Similar findings were reported by Withey et al. (1983) for several other halogenated hydrocarbons. The absorption rate and, therefore, peak blood levels will be inversely proportional to the volume of corn oil employed in oral dosing.

Sanzgiri et al. (1995) compared pharmacokinetics of carbon tetrachloride administered to fasted rats as a single bolus by gavage or by infusion over 2 hours. The doses, 17.5 and 179 mg/kg, were established by uptake measured in a 2-hour inhalation experiment (see Section 3.4.1.1). Carbon tetrachloride was rapidly absorbed in the gastrointestinal tract. Peak arterial blood concentrations were reached within 15 minutes of bolus administration and then declined, whereas infusion caused a steady increase over the 2-hour period. The peak concentrations were higher for the bolus group than for the infusion group.

3.4.1.3 Dermal Exposure

Carbon tetrachloride is significantly absorbed through the skin of humans, though less readily than from the lung or gastrointestinal tract. When volunteers immersed their thumbs in undiluted carbon tetrachloride for 30 minutes, carbon tetrachloride was detected in the alveolar air of each subject within 10 minutes, indicating relatively rapid percutaneous absorption (Stewart and Dodd 1964). The alveolar concentration of carbon tetrachloride rose steadily thereafter and peaked by about 30 minutes postexposure. The authors estimated that immersion of both hands in liquid carbon tetrachloride for 30 minutes would yield an exposure equivalent to breathing 100–500 ppm for 30 minutes. The investigators noted that the amount of carbon tetrachloride that can penetrate human skin appeared to be related to the method of application, the duration and area of skin exposure, and the type of skin exposed.

Studies in animals confirm that liquid carbon tetrachloride is absorbed through the skin (Jakobson et al. 1982; Morgan et al. 1991; Tsuruta 1975). The rate of uptake is high enough (54 nmol/min/cm^2 in mice) that absorbed doses may be comparable to the doses absorbed from relatively high levels of carbon tetrachloride in air (Tsuruta 1975). Uptake kinetics are linear only for a short time (about 30 minutes), after which blood levels tend to decrease (Jakobson et al. 1982; Morgan et al. 1991). This is probably due to local vasoconstriction in the exposed skin area. During the course of a 24-hour exposure (2 mL/3.1 cm^2 skin), rats absorbed 27% (0.54 mL) of the applied neat solution, whereas >99% of the carbon tetrachloride in 110–648 µg/mL aqueous solutions (approximately one-third to completely saturated) was absorbed (Morgan et al. 1991). Rather broad peak blood concentrations of approximately 8–70 ng/mL were observed 2–8 hours into the exposure period. In monkeys, the dermal absorption of radioactive carbon tetrachloride vapor at concentrations of 485 or 1,150 ppm over a period of 240 or 270 minutes, respectively, was negligible, as measured in samples of blood and expired air (McCollister et al. 1951).

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

No studies were located regarding distribution in humans after inhalation exposure to carbon tetrachloride.

Inhalation studies in monkeys (McCollister et al. 1951), rats (Benson et al. 2001; Dambrauskas and Cornish 1970; Paustenbach et al. 1986a, 1986b; Sanzgiri et al. 1997), and hamsters and mice (Benson et al. 2001) reveal that the highest carbon tetrachloride concentrations occur in fat, and in organs or tissues with high fat content such as bone marrow, liver, brain, and kidney. In rats exposed to 1,000 ppm for 2 hours (receiving a dose of 179 mg/kg), the maximal concentration of carbon tetrachloride was reached within 30 minutes (the earliest timepoint) in the liver, kidney, lung, brain, heart, muscle, spleen, and gastrointestinal tract, and by 240 minutes in fat (Sanzgiri et al. 1997). The area under the tissue concentration versus time curve (AUC) for the first 30 minutes of exposure was 322, 460, and 710 μg per minute/mL, respectively, for the liver, brain, and fat. The half-life of clearance from different organs (evaluated over 24 hours) ranged from 204 minutes for the kidney to 665 minutes for fat. Through the use of a low temperature whole-body autoradiographic technique, Bergman (1983) observed a particularly high uptake of ^{14}C-carbon tetrachloride into the white matter of brain, spinal cord, and spinal nerves in rats exposed by inhalation. Considerably lower levels were found in the kidney, lung, spleen, muscle, and blood.

Immediately following exposure to 20 ppm ^{14}C-labeled carbon tetrachloride vapor for 4 hours, the proportion of the initial body burden as carbon tetrachloride equivalents (CE) present in the major tissues was 30% for rats and hamsters and 40% for mice (Benson et al. 2001). The CE concentrations at that time were highest in the liver of mice and hamsters but were highest in fat for rats; 48 hours later, CE concentrations in all three species were highest in the liver. Clearance of CEs from various tissues was characterized as being best described by single- or two-component negative exponential functions. Clearance of CEs from the blood was complete within 48 hours and was described by a single-component function for all three species. The half-life for clearance ($T_{1/2}$) from blood was shortest for rats (1.8 hours) and longest for hamsters (23 hours). Clearance of CEs from the lung was also described by a single-component function for all three species, but was only about 80% complete after 48 hours; the $T_{1/2}$ ranged from 7 hours for rats to 17 hours for mice. Clearance of CEs from the liver in hamsters was complete and best described by a single-component function; the $T_{1/2}$ was 33 hours. In rats and mice, clearance from the liver was best described by a two-component function; a large fraction was cleared

with a $T_{1/2}$ of 3 hours and the remainder cleared with a $T_{1/2}$ of 35 hours. Clearance of CEs from the kidney in rats was complete and best described by a two-component function. In mice and hamsters, the $T_{1/2}$ for clearance from the kidney for the largest fraction (70–80%) of carbon tetrachloride was <10 hours, but no additional clearance occurred up to 48 hours.

3.4.2.2 Oral Exposure

No studies were located regarding distribution in humans after oral exposure to carbon tetrachloride.

Studies of the time-course of tissue distribution in male rats given oral doses of carbon tetrachloride reported that concentrations in the blood, striated muscle, brain, and liver were maximal 2 hours after dosing (Marchand et al. 1970). The peak concentrations in the liver and brain were significantly higher than in the muscle and blood. Peak levels in the fat were not reached until 5.5 hours post dosing, at which time they were more than 50-fold greater than peak blood levels. A similar time-course of tissue deposition of carbon tetrachloride has been observed in female rats (Teschke et al. 1983) and rabbits (Fowler 1969) dosed orally with carbon tetrachloride. Higher carbon tetrachloride levels were found consistently in the liver than in the brain of rats dosed orally (Marchand et al. 1970; Watanabe et al. 1986). This may be because carbon tetrachloride absorbed from the gastrointestinal tract enters the portal circulation, which initially passes through the liver. A significant proportion of the carbon tetrachloride is likely taken up from the portal blood during the first pass, resulting in the high liver levels following ingestion. One week after exposure to ^{14}C-carbon tetrachloride, the concentrations of radiolabel (expressed as mmol carbon tetrachloride/g tissue) were about 1.5 in plasma, 5–6.5 in soleus and white vastus lateralis muscle, 8 in liver, 10 in kidney and diaphragm, and 13 in adipose tissue (Weber et al. 1992). It is interesting to note that phenobarbital pretreatment, often used to hasten or intensify the toxic effects of carbon tetrachloride exposure, was found not only to nearly double the amount of radiolabel retained in the examined tissues, but also to significantly alter its distribution. Liver, kidney, and plasma concentrations were elevated to 600, 350, and 150% of their respective control (carbon tetrachloride alone) levels, while the muscle, diaphragm, and adipose levels were reduced to 40–70%. This observation is consistent with higher levels of the administered dose being metabolized (largely in the liver) and subsequently entering the carbon pool.

In rats receiving a dose of 179 mg/kg by infusion over 2 hours, the maximal concentration of carbon tetrachloride was reached by 120 minutes in the liver, kidney, and heart, 150 minutes in the brain, muscle, and spleen, 180 minutes in lung, and by 360 minutes in fat (Sanzgiri et al. 1997). The AUC for the first

30 minutes of exposure was 3, 28, and 157 µg per minute/mL, respectively, for the liver, brain, and fat in infused rats. Absorption of carbon tetrachloride was more rapid and organ concentrations of carbon were higher in rats that received the same dose as a single bolus by gavage. The maximal concentration was reached by 1 minute in the liver, 5 minutes in the kidney, heart, and spleen, 15 minutes in lung and brain, 60 minutes in muscle, and 120 minutes in fat. The AUC for the first 30 minutes was 680, 423, and 306 µg per minute/mL, respectively, for the liver, brain, and fat in the bolus-treated rats. The authors indicated that the bolus-delivery resulted in high 30-minute AUC values because the capacity of first-pass hepatic and pulmonary elimination was exceeded. The half-life of clearance from different organs (based on the AUC over 24 hours) ranged from 190 minutes for the kidney to 358 minutes for fat in the infused rats and from 278 minutes for the kidney to 780 minutes for fat in the bolus-treated rats.

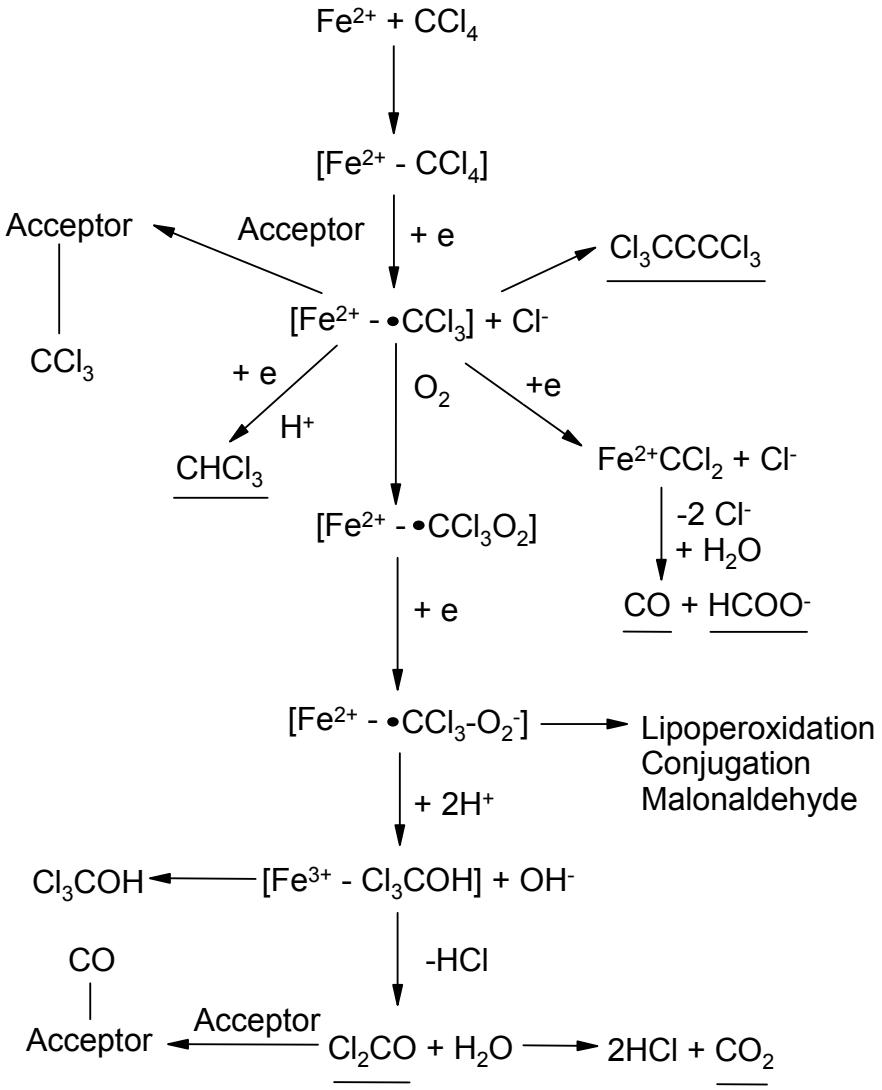
3.4.2.3 Dermal Exposure

No studies were located regarding distribution in humans or animals after dermal exposure to carbon tetrachloride.

3.4.3 Metabolism

The metabolism of carbon tetrachloride in humans has not been investigated, but a great deal of information is available from studies in animals. Pathways of carbon tetrachloride metabolism are illustrated in Figure 3-3, and metabolites that have been identified are underlined. Bioactivation of carbon tetrachloride proceeds by cytochrome P-450 dependent reductive dehalogenation (Sipes et al. 1977). CYP2E1 is the primary enzyme responsible for metabolizing carbon tetrachloride in humans at environmentally relevant concentrations, but others, particularly CYP3A, are also involved at higher concentrations (Zangar et al. 2000). Studies with CYP2E1 genetic knockout mice *(cyp2e1$^{-/-}$)* demonstrated that hepatic toxicity of carbon tetrachloride in mice is entirely dependent on CYP2E1 (Wong et al. 1998). A large body of experimental data indicates that the first step involves homolytic cleavage of one carbon chlorine bond in carbon tetrachloride to yield chloride ion and the trichloromethyl radical (Lai et al. 1979; Poyer et al. 1978). Anerobically, the trichloromethyl radical may undergo several reactions, including (1) direct binding to microsomal lipids and proteins (Rao and Recknagel 1969); (2) addition of a proton and an electron to form chloroform (Glende et al. 1976; Uehleke et al. 1973); (3) dimerization to form hexachloroethane (Fowler 1969; Uehleke et al. 1973); and (4) further reductive dechlorination to form carbon monoxide (Wolf et al. 1977). Aerobically, trichloromethyl radical may be

Figure 3-3. Pathways of Carbon Tetrachloride Metabolism*



*Adapted from Shah et al. 1979. Fe^{2+} and Fe^{3+} denote the reduced and oxidized forms of cytochrome P-450, and brackets indicate an enzyme substrate complex. Electrons are donated from NADPH or NADH.

oxygenated by the mixed-function oxidase system to yield trichloromethanol, a precursor to phosgene. Hydrolytic cleavage of phosgene is likely the major pathway by which carbon dioxide is formed from carbon tetrachloride (Shah et al. 1979).

Investigations indicate that carbon tetrachloride is metabolized by a specific form of hepatic cytochrome P-450 (the 52,000 dalton component) (Castillo et al. 1992), which is rapidly destroyed during the metabolic process (Noguchi et al. 1982a, 1982b) and is the ethanol-inducible isoform P-450 CYP2E1 (Castillo et al. 1992). CYP2E1 may be lost by either a direct attack (i.e., covalent binding) of radicals on the cytochrome(s) (Manno et al. 1992; Vittozzi and Nastainczyk 1987), or highly localized lipid peroxidation resulting in detachment of P-450 proteins from the microsomal membranes. Cytochrome P-450 mediated homolytic cleavage of the carbon-chlorine bond in carbon tetrachloride is thought to be followed by hydrogen abstraction by the trichloromethyl radical at a methylene group of polyenic fatty acids in the microsomal lipids, thus forming organic free radicals. These organic free radicals then rapidly react with molecular oxygen, leading to the formation of organic peroxy free radicals and eventually organic peroxides (Rao and Recknagel 1969; Recknagel 1967; Recknagel and Glende 1973). The unstable organic peroxides cleave homolytically to form new free radicals, which attack methylene groups of neighboring polyenic lipids in the membrane. This autocatalytic process occurs very rapidly; hepatic microsomal lipid peroxidation is more than half of its maximum value at 5 minutes, and is complete within 15 minutes after oral administration of carbon tetrachloride to fasted rats (Rao and Recknagel 1968). Lipid peroxidation can contribute to breakdown of membrane structure and loss of organelle and cell functions. Connor et al. (1986) conducted a study in which they detected the trichloromethyl radical and a second free radical, the carbon dioxide anion radical, by electron spin resonance spectroscopy in liver perfusate and in urine of female rats. Adducts of both radicals have also been detected in blood of male rats (Reinke and Janzen 1991).

Cytochrome P-450 from rat or human liver microsome preparations is inactivated when incubated anaerobically with carbon tetrachloride in the presence of NADPH and an oxygen-scavenging system (Manno et al. 1988, 1992). Inactivation involved destruction of the heme tetrapyrrolic structure, and followed pseudo first-order kinetics with fast and slow half-lives of 4.0 and 29.8 minutes. When compared with rat liver microsomes, the human preparations were 6–7 times faster at metabolizing carbon tetrachloride and only about one-eighth as susceptible to self-destructing ("suicidal") inactivation (about 1 enzyme molecule lost for every 196 carbon tetrachloride molecules metabolized).

The rate of carbon tetrachloride metabolism *in vivo* has been estimated primarily by indirect methods. Male rats were exposed to carbon tetrachloride vapor in a desiccator jar with a recirculating atmosphere. The decline in the chamber concentration was monitored over time as the index of carbon tetrachloride uptake into the animals (Gargas et al. 1986). The shapes of the uptake curves were a function of tissue partition coefficients and the metabolism of carbon tetrachloride. The uptake kinetics of carbon tetrachloride were accurately described by a physiological pharmacokinetic model with a single, saturable metabolic pathway. The maximum rate of reaction (Vmax) was calculated to be 0.14 mg/hour (0.62 mg/kg/hour), while the half-maximum rate concentration of carbon tetrachloride (Km, the Michaelis-Menten constant) was calculated to be 1.62 μM (0.25 mg/L). Carbon tetrachloride was metabolized more slowly than other halocarbons studied (methyl chloroform, 1,1-dichloroethylene, bromochloromethane). Another indirect method was evaluated for estimating the rate of carbon tetrachloride metabolism in male rats, based on arterial blood:inhaled air concentration ratios (Uemitsu 1986). Results of this study suggest that carbon tetrachloride metabolism was limited by the rate of blood perfusion of the liver at concentrations below 100 ppm, and was saturated at concentrations above 100 ppm. The estimated Vmax was 2.8 mg/kg/hour. The rate of metabolism gradually decreased during the exposure period, apparently the result in carbon tetrachloride-induced loss of cytochrome P-450.

Based on comparative PBPK modeling, which incorporated *in vivo* and *in vitro* data, Thrall et al. (2000) calculated that the rates of metabolism (V_{max}/K_m) by milligrams of liver protein differed across species, with hamster > mouse > rat > human. The human *in vivo* metabolic rates for carbon tetrachloride were estimated as 1.49 mg/hour/kg body weight (V_{max}) and 0.25 mg/L for K_m.

A study was conducted in which the extent of metabolism of ^{14}C-carbon tetrachloride in rats was assessed by measuring the amounts of unchanged carbon tetrachloride, carbon dioxide, and chloroform exhaled in the breath, ^{14}C-metabolite excreted in urine and feces, and ^{14}C-metabolite bound to liver macromolecules within a 24-hour period post oral dosing (Reynolds et al. 1984). The major metabolite in this study was carbon dioxide at all dose levels, ranging from 85% of total metabolites recovered at 15 mg/kg to 63% at 4,000 mg/kg. The modest 22% (from 85 down to 63%) reduction in carbon dioxide production when the dose is increased 28-fold (15 versus 4,000 mg/kg) suggests that excess amounts of P-450 are available in the liver for metabolism of carbon tetrachloride. Intermediate amounts of nonvolatile ^{14}C-labeled material were recovered from the urine and feces, although none of the metabolites were identified by these investigators. About 2–4% of the label was found covalently bound to liver macromolecules. The relative amount of chloroform formed depended on dose, with chloroform being the least abundant metabolite formed at the lowest dose, but the second most abundant metabolite at the highest dose. As

the dose of carbon tetrachloride increased, the fraction of the dose recovered decreased for each metabolite except chloroform. A major change in the overall extent of carbon tetrachloride metabolism occurred as the dose was increased from 15 to 46 mg/kg, the nature of which suggests that the oxidative metabolism of carbon tetrachloride was saturated and/or impaired by destruction of cytochrome P-450 in this dosage range. The fraction recovered in the expired air as unchanged carbon tetrachloride increased from 20 to 80% of the administered dose, and the peak carbon tetrachloride exhalation rate increased 40-fold. Thus, this study indicated that when oxidative metabolism of carbon tetrachloride was saturated or inhibited, more of the parent chemical was exhaled and increased amounts of chloroform were formed by a reductive pathway. Low levels of carbon tetrachloride metabolism to CO_2 were also indicated by other studies showing that 6 hours after intraperitoneal injection of 128–159 mg/kg carbon tetrachloride to rats or gerbils, <1% (approximately 0.2% for rats, and 0.7% for gerbils) of the dose had been expired as CO_2, while approximately 80–90% had been expired as unchanged carbon tetrachloride (Cai and Mehendale 1990; Mehendale and Klingensmith 1988; Young and Mehendale 1989).

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

Little quantitative information was located regarding the amount or fraction of absorbed carbon tetrachloride that is subsequently excreted in air, urine, or feces in humans exposed by inhalation. Studies of the rate of excretion of carbon tetrachloride in the expired air were conducted in a worker who had been exposed to carbon tetrachloride vapors for several minutes (Stewart et al. 1965). The concentration of carbon tetrachloride appeared to decline exponentially in a biphasic manner, with an initial half-life of <1 hour, and a second-phase half-life of about 40 hours. Roughly similar results were observed in several volunteers who breathed carbon tetrachloride for 1–3 hours, where the half-life of carbon tetrachloride in expired air over the first several hour period after exposure was <1 hour (Stewart et al. 1961).

Studies in animals indicate about 30–40% of an inhaled dose of carbon tetrachloride is excreted in expired air and about 32–62% is excreted in feces (McCollister et al. 1951; Paustenbach et al. 1986a). Relatively low amounts are excreted in urine. Nearly all of the material in expired air is parent carbon tetrachloride, with only small amounts of carbon dioxide. The identity of the nonvolatile metabolites in feces and urine was not determined.

During the 48 hours following nose-only inhalation exposure to 20 ppm ^{14}C-labeled carbon tetrachloride vapor for 4 hours, rats, mice and hamsters eliminated 65–83% of the initial body burden of ^{14}C activity as

CO_2 or volatile organic compounds in exhaled breath (Benson et al. 2001). Elimination in expired air was described as a single-order negative exponential function. Elimination half-times for carbon tetrachloride equivalents (CEs) in exhaled breath were 4.3, 0.8, and 3.6 hours for volatile organic compounds and 7.4, 8.8, and 5.3 hours for CO_2 for rats, mice, and hamsters, respectively. The fraction of the initial body burden of CEs eliminated in urine and feces combined was <10% in rats and >20% in mice and hamsters.

As in humans, the rate of carbon tetrachloride excretion in rats appears to be biphasic, with an initial half-life value of 7–10 hours (Paustenbach et al. 1986a). The rapid phase was judged to reflect clearance from blood, while the slower phase was related to clearance from fatty tissue and metabolic turnover of covalent adducts (Paustenbach et al. 1988). In support of this, exposure for longer periods of time led to higher concentrations of carbon tetrachloride in fat and a decreased rate of clearance (Paustenbach et al. 1986a, 1986b, 1988).

3.4.4.2 Oral Exposure

The concentration of carbon tetrachloride was measured in the expired air of a person who swallowed a large amount of carbon tetrachloride (Stewart et al. 1963). Excretion in expired air was found to decrease exponentially in a biphasic or multiphasic fashion, but no quantitative estimate of the elimination half-life of carbon tetrachloride or of the fraction of the dose excreted by this pathway was provided. Visual inspection of their graphed data suggests very approximate half-lives of less than several hours initially, 40 hours (75–150 hours post exposure), and 85 hours (300–400 hours post exposure).

A detailed investigation of carbon tetrachloride excretion was performed in rats exposed by gavage to a range of doses (Reynolds et al. 1984). At doses of 50 mg/kg or higher, most of the dose (70–90%) was recovered in expired air as unchanged carbon tetrachloride. Lower amounts were recovered as expired carbon dioxide or chloroform, or as nonvolatile metabolites in feces or urine. As would be expected for a saturable or self-destructing metabolic system, the proportion of each dose recovered as metabolites tended to decrease as the dose increased. For example, 12% of the lowest dose (0.15 mg/kg) was recovered as carbon dioxide, while only 0.7% of the highest dose (4,000 mg/kg) was recovered as carbon dioxide. The time-course of excretion also depended on dose, tending to become slower as doses increased. For example, the half-life for exhalation of carbon tetrachloride was 1.3 hours at a dose of 50 mg/kg, but was 6.3 hours at a dose of 4,000 mg/kg. This is consistent with the concept that an increased proportion of a dose enters fat as the dose level increases, with clearance from fat being slower than from blood and other tissues. Increased hepatotoxicity in the form of greater cytochrome P-450

destruction (and thus reduced carbon tetrachloride metabolism) may also be a significant factor. Studies evaluating the rate of excretion over the first 12 hours described a one-compartment model, but did not deduce that a two-compartment model was inappropriate (Reynolds et al. 1984). Approximately 24 hours after receiving an oral dose of 3,985 mg/kg, rats were observed to excrete elevated levels of various lipid peroxidation products (formaldehyde, acetaldehyde, malondialdehyde, and acetone) in their urine, presumably as a result of carbon tetrachloride-induced oxidative stress (Shara et al. 1992).

3.4.4.3 Dermal Exposure

Carbon tetrachloride was rapidly excreted in expired air of volunteers who immersed their thumbs in liquid carbon tetrachloride (Stewart and Dodd 1964). The half-life of expiration was about 30 minutes, but no quantitative estimate of the fraction of the absorbed dose excreted in air was performed. No studies were located regarding excretion in animals after dermal exposure to carbon tetrachloride.

3.4.4.4 Other Routes of Exposure

After what was described as either intragastric or intraduodenal administration of carbon tetrachloride to rats under various conditions, evidence from electron paramagnetic resonance experiments using phenyl-N-t-butyl nitrone as a spin trap suggested that trichloromethyl free-radical adducts are secreted into the bile without being concentrated, and in concentrations which reflect those concurrently found in the liver (Knecht and Mason 1991). Expressed in arbitrary concentration units, spin-trap-bound adduct quantities found in the liver, in the bile, and liver/bile concentration ratios under the various experimental conditions were as follows: carbon tetrachloride alone (93, 28, 3.4 ratio), carbon tetrachloride plus hypoxia (161, 50, 3.2 ratio), carbon tetrachloride with phenobarbital pretreatment (118, 69, 1.7 ratio), and carbon tetrachloride with intravascular infusion of the bile salt dehydrocholate to double the bile flow rate (85, 13, 6.8 ratio). Taken together, these results from conditions that vary bile flow or reductive metabolic generation of free radical seem to indicate that carbon tetrachloride free-radical adducts are secreted rather than merely diffused into bile, and in amounts proportional to their generation in the liver. The drop in liver/bile ratio observed with phenobarbital pretreatment (from 3.4 to 1.7) was attributed to the liver's phenobarbital-enhanced ability to destroy many of the induced free-radical adducts. These results are supported by findings in bile duct-cannulated rats and in perfused rat liver systems, where spin-trapped free-radical adducts were observed in bile, but not in blood or urine (Hughes et al. 1991).

As noted above, within 6 hours of intraperitoneally injecting rats or gerbils with 128–159 mg/kg of carbon tetrachloride, 80–90% of the administered dose was expired as unchanged carbon tetrachloride, while less than 1% was expired as CO_2 (Cai and Mehendale 1990; Mehendale and Klingensmith 1988; Young and Mehendale 1989). After rats were injected intraperitoneally with 3 mL carbon tetrachloride per kg body weight, volatile carbonyl compounds released into expired air over 24 hours were evaluated by gas chromatography (Dennis et al. 1993). Injected rats exhaled significantly higher levels of acetone and a compound tentatively identified as formyl chloride than control rats; the amounts of acetaldehyde and formaldehyde were not significantly different in the two groups.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-

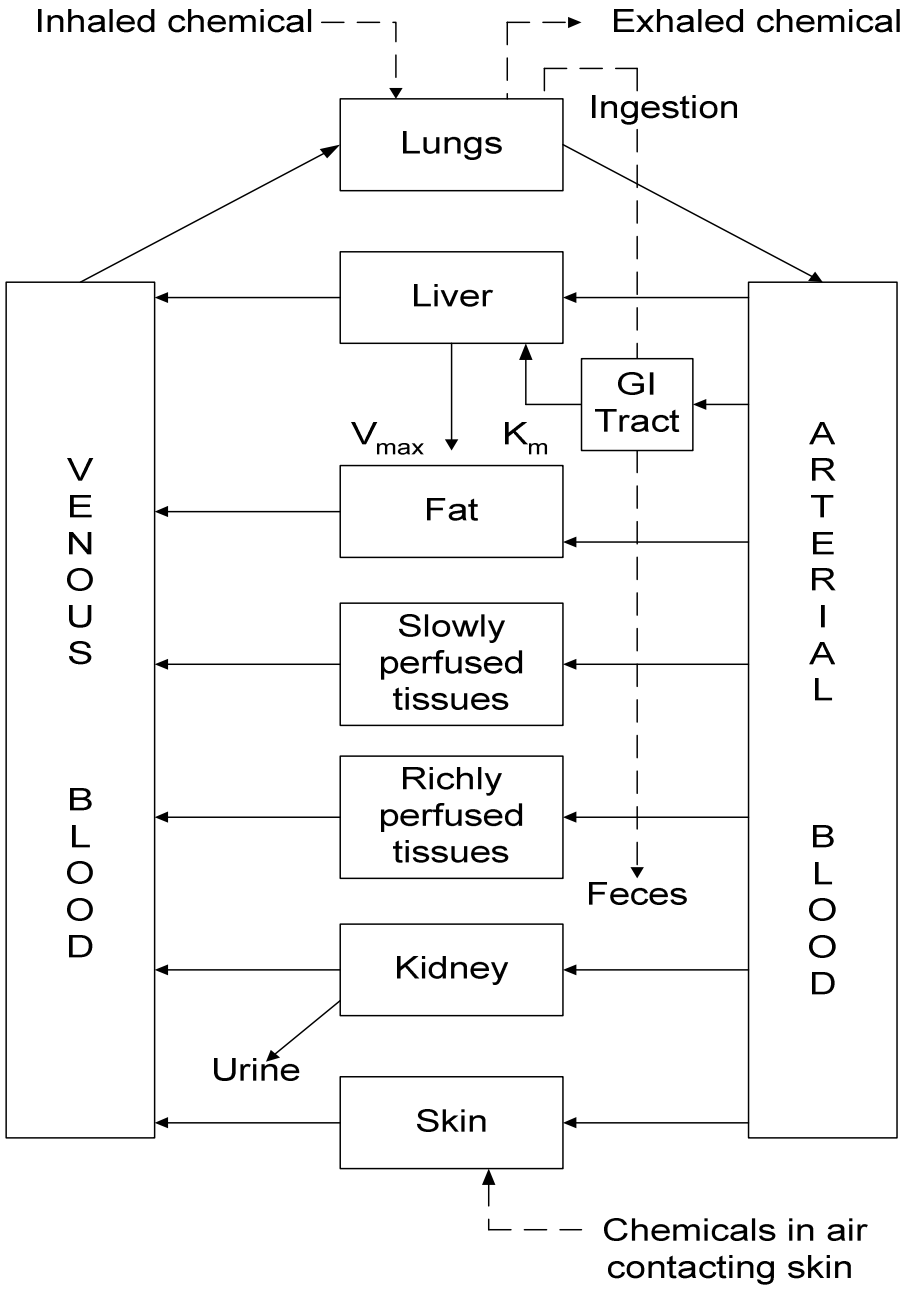
specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

A detailed physiologically based pharmacokinetic model (Figure 3-5) has been developed that describes the metabolism of carbon tetrachloride following inhalation exposure (Paustenbach et al. 1988). The model was based on and validated against a previous study in rats in which 1–2 weeks of inhalation exposure to 100 ppm ^{14}C-labeled carbon tetrachloride for 8–11.5 hours/day, 4–5 days/week apparently resulted in 40–60% of the absorbed dose being metabolized (Paustenbach et al. 1986a). The model incorporated partition characteristics of carbon tetrachloride (blood:air and tissue:blood partition coefficients), anatomical and physiological parameters of the test species (body weight, organ weights, ventilation rates, blood flows), and biochemical constants (V_{max} and K_m) for carbon tetrachloride metabolism. The model accurately predicted the behavior of carbon tetrachloride and its metabolites, both the exhaled unmetabolized parent compound and $^{14}CO_2$ and the elimination of radioactivity in urine and feces. In agreement with other studies (Gargas et al. 1986; Uemitsu 1986), Paustenbach et al. (1988)

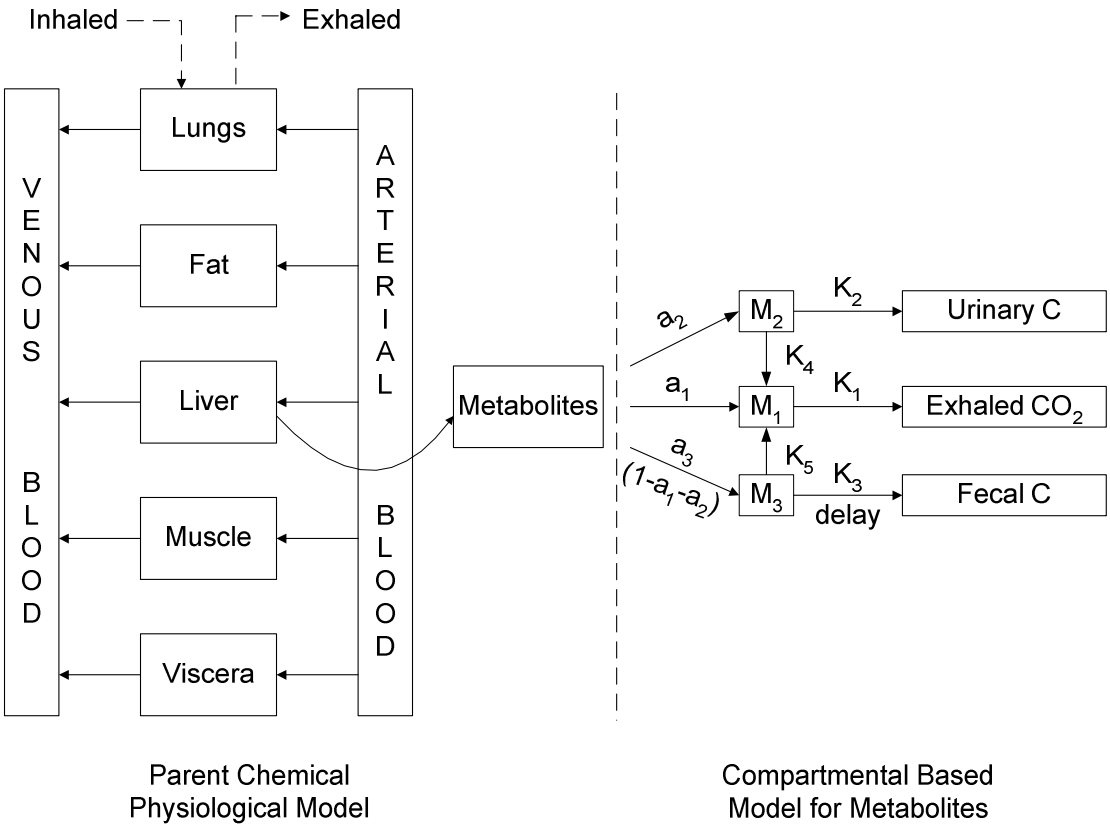
Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physicologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Figure 3-5. Physiologically Based Pharmacokinetic Model for Inhaled Carbon Tetrachloride*



*Adapted from Paustenbach et al. 1988

found that metabolism was best described as a single saturable pathway, with a V_{max} of 0.65 mg/kg/hour and a K_m of 0.25 mg/L. Metabolites were partitioned in the model to three compartments: the amounts to be excreted in the breath (as $^{14}CO_2$), urine, and feces. Of total carbon tetrachloride metabolites, 6.5% was excreted as CO_2, 9.5% was excreted in urine, and 84.0% was excreted in feces. Based on this model, the authors estimated that about 4% of initially metabolized carbon tetrachloride is converted directly to carbon dioxide and is promptly excreted, while the remainder forms adducts with proteins and other cellular molecules. These adducts are then degraded with a half-life of about 24 hours, and the products are excreted mainly in the urine and feces, with small amounts eliminated as carbon dioxide. The amount of carbon tetrachloride metabolized is limited by the saturable enzyme system, with high exposures (e.g., 100 ppm) leading to saturation within a short time. Following cessation of exposure, considerable metabolism may occur as carbon tetrachloride emerges from fatty tissue. The model successfully described elimination using a V_{max} of 0.65 mg/kg/hour and a K_m of 0.25 mg/L. The model was scaled up to predict the expected behavior of carbon tetrachloride in monkeys and humans. The results were consistent with data collected by McCollister et al. (1951) and Stewart et al. (1961). The earlier study by Paustenbach et al. (1986) showed that rats did not have significant day-to-day accumulations in the blood or fat following repeated exposure to 100 ppm for 8 or 11.5 hours/day; this was accurately described in the model. In contrast, humans exposed to 5 ppm for 8 hours/day would be expected to show day-to-day increases in fat because of physiological differences.

Thrall et al. (2000) adapted the model of Paustenbach et al. (1988) to compare the metabolism of carbon tetrachloride in male rats, mice, and hamsters exposed to 40–1,800 ppm in a recirculating closed-chamber gas-uptake system. For each species, an optimal fit of the uptake curves was obtained by adjusting the metabolic constants V_{max} (capacity) and K_m (affinity) using the model. The mouse had a slightly higher capacity and lower affinity for metabolizing carbon tetrachloride than the rat, whereas the hamster had a higher capacity and lower affinity than either the rat or mouse. A comparison of V_{max}/K_m normalized for milligrams of liver protein (L/hour/mg) indicated that hamsters metabolize more carbon tetrachloride than rats or mice. The species comparisons were evaluated against toxicokinetic studies conducted in animals exposed by by nose-only inhalation to 20 ppm ^{14}C-labeled carbon tetrachloride for four hours. Rats eliminated a lower fraction of the dose as metabolites and more as parent compound compared to mice or hamsters. The use of the model was expanded to include *in vitro* constants using liver microsomes from rat, mouse, hamster, and human in order to estimate *in vivo* metabolic rates for humans: a V_{max} of 1.49 mg/hour/kg body weight and a K_m of 0.25 mg/L. Normalizing the rate of metabolism (V_{max}/K_m), the rate of metabolism differed across species, with hamster > mouse > rat > human.

Yoshida et al. (1999) estimated rates of absorption of carbon tetrachloride and three trihalomethanes in low-level inhalation exposures by rats using a pharmacokinetic analysis. A three-compartment model, consisting of a tank with barium chloride to trap the chemical, the exposure chamber, and the rat, was employed for carbon tetrachloride, which was injected into the chamber. The model estimated that the amounts of carbon tetrachloride metabolized by rats in μmol/hour/kg were 0.000053, 0.0053, and 0.53 for exposures at 1 ppb, 10 ppb, and 10 ppm, respectively.

Semino et al. (1997) adapted the model of Paustenbach et al. (1988) to develop a PBPK model to describe the oral uptake of carbon tetrachloride administered to male Fischer 344 rats in corn oil or 0.25% Emulphor, an aqueous vehicle. The gastrointestinal model used a series of subcompartments with an absorption constant (K_a, L/hour), a bioavailability term (A, unitless), and a compartment emptying time (T, hours). The model was optimized by varying the values of the constants for the experimental data. Higher values of K_a and A were needed to fit data from aqueous gavage compared to that for corn oil. The model provided precise fits of multipeak blood and exhaled breath carbon tetrachloride concentration-time profiles. A pulsatile pattern noted following corn oil gavage was attributed to discontinuous emptying of the stomach into the small intestine. Initial absorption of the bolus occurs rapidly in the stomach, especially for aqueous vehicles; subsequently, stomach absorption slows and uptake from the small intestine determines the absorption profile.

Gallo et al. (1993) developed a PBPK model for blood concentration of carbon tetrachloride in rats following intravenous delivery in aqueous polyethylene glycol 400. Subsequently, absorption input functions were added to the model to describe blood concentration profiles resulting from administration of 25 mg carbon tetrachloride per kg body weight alone, in aqueous vehicles (water or 0.25% Emulphor emulsion), or in corn oil. Absorption was 91.9% for administration in water, 85.4% in Emulphor, 62.8% for the pure compound, and 93.1% for administration in corn oil. A pulsatile pattern was obtained for absorption in corn oil.

Andersen et al. (1996) developed a pharmacokinetic model to calculate the concentration of carbon tetrachloride in microsomal suspensions from male Fischer 344 rats under anerobic conditions. Dose-response curves revealed a nonlinear, biphasis appearance of trichloromethane. One experiment compared microsomes from fasted or unfasted rats; fasting did not alter the shape of the dose-response curve, but increased the production of trichloromethane in microsomes.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. As a small volatile haloalkane, carbon tetrachloride diffuses passively across cell membranes, leading to rapid absorption from the lungs and gastrointestinal tract into the circulatory system (Sanzgiri et al. 1995, 1997). Pulmonary absorption is ventilation limited.

Distribution. Being somewhat lipophilic, absorbed carbon tetrachloride diffuses from the blood to the liver, kidney, brain, and other organs and accumulates in adipose tissue. Following absorption by the gastrointestinal tract, a first-pass effect is apparent through the liver, where carbon tetrachloride is biotransformed and adducts are formed from reactive metabolites binding to cell macromolecules. Clearance of unmetabolized carbon tetrachloride is limited by passive diffusion; the rate of clearance is slowest for adipose tissue compared to internal organs (Benson et al. 2001; Sanzgiri et al. 1997). Delivery of carbon tetrachloride as a single bolus can exceed first-pass hepatic and pulmonary elimination, resulting in higher blood levels and more severe hepatic injury compared to gradual delivery of the same dose over a longer period of time (Sanzgiri et al. 1997).

Metabolism. Carbon tetrachloride is primarily metabolized in tissues that express CYP2E1. The metabolic pathways are described in detail in Section 3.4.3 and depicted in Figure 3-3.

Excretion. In humans and animals, carbon tetrachloride is eliminated by passive diffusion primarily through exhaled breath, with a smaller fraction eliminated in urine and feces (Benson et al. 2001; Thrall et al. 2000).

3.5.2 Mechanisms of Toxicity

Unmetabolized carbon tetrachloride, as a volatile halogenated alkane, depresses the central nervous system. All other toxic effects of carbon tetrachloride are related to its biotransformation catalyzed by cytochrome P-450 dependent monooxygenase, specifically CYP2E1 (Azri et al. 1991; Hughes et al. 1991; Wong et al. 1998; Zangar et al. 2000). The liver and kidney (especially in humans) are especially vulnerable because of the abundance of CYP2E1, which is also present in the respiratory and nervous systems. Considerable data are available for hepatic toxicity, but similar cellular damage would be expected in other tissues with a high abundance of CYP2E1. There is considerable evidence that hepatic injury produced by carbon tetrachloride is mediated by two major processes resulting from bioactivation

in the endoplasmic reticulum and mitochondria of centrilobular hepatocytes, which have the highest concentration of CYP2E1 (Buhler et al. 1992): haloalkylation of cellular macromolecules by reactive metabolites (trichloromethyl free radical or trichloromethyl peroxyl free radical) and lipid peroxidation, which impairs cellular functions dependent on membrane integrity (Weber et al. 2003). Both haloalkylation and lipid peroxidation contribute to loss of cellular functions and subsequent cell death as discussed in greater detail in the following paragraphs. In response to parenchymal cell damage, perisinusoidal cells may be stimulated to release extracellular matrix proteins (type-I collage) that contribute to hepatic fibrogenesis, which is largely mediated by hepatic macrophages (Kupffer cells) (Belyaev et al. 1992; Ishiki et al. 1992; Johnson et al. 1992; Muriel and Escobar 2003). Kupffer cells activated by carbon tetrachloride release tumor necrosis factor-alpha (TNF-alpha), nitric oxide, transforming growth factor-beta (TGF-beta) (Date et al. 1998), and interleukins (IL) -1, -6 and -10 (Weber et al. 2003). TNF-alpha elicits an inflammatory response and may generate aptoptosis or contribute to the development of steatosis in heptocytes (Morio et al. 2000). TNF-alpha may also stimulate genes involved in hepatic mitogenesis (Bruccoleri et al. 1997). Nitric oxide generally protects against apoptopic tissue damage (Muriel 1998), but can also react with the O_2^- radical (formed during carbon tetrachloride-induced oxidative stress) to form an aggressive peroxynitrite radical, resulting in more severe hepatic injury (Morio et al. 2001; Weber et al. 2003). Lipid peroxidation may be at least partially independent of cytochrome P-450, as iron-dependent peroxidation occurred in cultured mammalian cells even in the presence of P-450 inhibitors (Dickens 1991). While carbon tetrachloride-induced liver damage was mitigated by treatment with allopurinol, an inhibitor of xanthine oxidase (a free radical-generating enzyme), prolonged administration of the free radical scavenger superoxide dismutase actually aggravated hepatocellular damage (Dashti et al. 1992).

Hepatic microsomal lipid peroxidation damages cellular functions by disturbing the integrity and hence the function of membranes and bycovalent binding of reactive intermediates. The trichloromethyl radical is sufficiently reactive to bind covalently to CYP2E1, a process referred to as the "suicidal inactivation" of CYP2E1 (Fujii 1997; Manno et al. 1988, 1992). It is also possible that reactive intermediates formed during the process of lipid peroxidation contribute to the loss of CYP2E1. Nevertheless, it is still not clear how these initial events are related to subsequent triglyceride accumulation, polyribosomal disaggregation, depression of protein synthesis, cell membrane breakdown and eventual death of the hepatocytes. Carbon tetrachloride can inhibit triglyceride secretion from hepatocytes in the absence of lipid peroxidation, and polyribosomal dissociation and decreased protein synthesis can occur when no ^{14}C-labelled carbon tetrachloride has been incorporated into ribosomal fractions (Waller et al. 1983). When rats were pretreated with a chemical that reduced lipid peroxidation by 85%, only small recoveries

from carbon tetrachloride-induced decreases in hepatocellular viability, cytochrome P-450 content, aniline hydroxylase activity, and carbon tetrachloride metabolism capacities were observed (Kostyuk and Potapovich 1991). This suggests that free radical binding to critical cellular macromolecules (e.g., microsomal oxidation system enzymes) may be more critical for these effects than lipid peroxidation. On the other hand, inhalation exposure to carbon tetrachloride produced a direct correlation between lipid peroxidation and proline hydroxylase (a collagen biosynthetic enzyme) in rats, and dietary zinc supplementation was associated with decreases in lipid peroxidation, collagen deposition, and proline hydroxylase activity, together with an increase in collagenase activity (Camps et al. 1992).

Another factor that may be of importance in carbon tetrachloride-induced hepatotoxicity is the perturbation of normal cellular calcium homeostasis following exposure. A number of studies have reported data that suggest carbon tetrachloride exposure inhibits the capacity of the hepatocyte endoplasmic reticulum or microsomal fraction to sequester (or keep sequestered) calcium, under either *in vivo* (Kodavanti et al. 1993; Long and Moore 1986a; Long et al. 1989; Lowrey et al. 1981b) or *in vitro* (Long and Moore 1987; Long et al. 1989; Lowrey et al. 1981a; Srivastava et al. 1990; Waller et al. 1983) exposure conditions. This inhibition of sequestration capacity is considered to be a key contributor to the rise in cytosolic calcium concentration that is generally observed following carbon tetrachloride exposure (e.g., Kodavanti et al. 1990b, 1993; Long and Moore 1987), and that is postulated to play a central role in the induced cytotoxicity. While some *in vivo* (Long and Moore 1986a) and *in vitro* (Srivastava et al. 1990) data suggest that carbon tetrachloride intoxication actually promotes the release of calcium to the cytosol from the endoplasmic reticulum or microsomes, other *in vivo* studies with carbon tetrachloride alone (Yamamoto 1990b) or in conjunction with chlordecone (Agarwal and Mehendale 1984a, 1984b, 1986) indicate that microsomal calcium content in fact rises, though generally to a lesser extent than cytosolic or total calcium content. Such microsomal increases presumably occur despite diminished calcium sequestration capacity. Studies have indicated that increased intracellular calcium may mediate cytotoxicity by activating phospholipase A2 (Chiarpotto et al. 1990; Glende and Recknagel 1991, 1992), which might contribute to irreversible plasma membrane damage. Elevated intracellular calcium may also be associated with elevated levels of phosphorylase and altered intracellular levels and distribution of calmodulin (Kodavanti et al. 1990), but was reported not to result in any DNA degradation—a potential result of calcium-activation of endonuclease activity (Long et al. 1989).

The finding that carbon tetrachloride is converted to reactive metabolites that bind to nuclear protein, lipids, and DNA may be relevant to the understanding of carbon tetrachloride carcinogenicity. Binding of radiolabel to liver cytoplasmic and nuclear proteins was found in Wistar rats and Swiss mice dosed with

^{14}C-carbon tetrachloride (Rocchi et al. 1973). Pretreatment of the animals with 3-methylcholanthrene (an inducer of cytochrome P-450 IA [P-448]) resulted in ^{14}C binding to hepatic DNA of mice, but not rats. Similarly, Diaz Gomez and Castro (1980a) found significantly greater ^{14}C binding to the liver DNA of A/J mice than to that of Sprague-Dawley rats given a tracer dose of ^{14}C-carbon tetrachloride. A/J mice are among the most susceptible of strains tested with respect to liver tumor induction by carbon tetrachloride. Administration of a high dose (3,200 mg/kg) of ^{14}C-carbon tetrachloride, having the same total radioactivity as the tracer dose, resulted in much more intensive binding to hepatic DNA. Presumably, the fewer reactive metabolites formed from the tracer dose react primarily with microsomal lipids and proteins in close proximity to their formation. With the higher dose, more ^{14}C-carbon tetrachloride can apparently reach the nucleus and be metabolically activated there, subsequently reacting with nuclear lipids, proteins, and DNA. This scenario receives support from the finding that highly purified rat liver nuclear preparations were able to anaerobically activate ^{14}C-carbon tetrachloride in the presence of an NADPH generating system (Diaz Gomez and Castro 1980b). Under microsome-mediated aerobic conditions, it was observed that ^{14}C-carbon tetrachloride bound more to histone than to nonhistone chromosomal proteins from livers of B6C3F$_1$ mice (Oruambo and Van Duuren 1987). These findings may be relevant to the understanding of carbon tetrachloride hepatocarcinogenicity, since reactive metabolites of carbon tetrachloride appear capable of binding to targets of putative relevance to cancer induction (chromosomal DNA and nucleosome proteins), and may even be generated within the nucleus itself. Since lipid peroxidation products such as malonaldehyde also have the ability to form adducts with DNA (Chaudhary et al. 1994; Chung et al. 2001; Wacker et al. 2001), it is possible that the genotoxic effect of carbon tetrachloride is partly indirect. Malonaldehyde-initiated tumors have been reported in Swiss mice (Shamberger et al. 1974). It is also worth noting that data from a variety of congenic mouse strains suggest that both the toxicity of, and recovery from, carbon tetrachloride exposure are under genetic control (an Ah gene, and H-2 genes) (Bhathal et al. 1983; Biesel et al. 1984). Despite some evidence for indirect genotoxicity of carbon tetrachloride, it appears that hepatic carcinogenicity in exposed rodents is directly related to the increase in cellular replication that occurs in response to hepatocyte lethality. Enhanced cellular replication increases the possibility that unrepaired DNA errors will become fixed mutations, possibly resulting in an initiated preneoplastic cell.

Interesting data from other studies illustrate that the hepatotoxic effects of carbon tetrachloride (or carbon tetrachloride plus chlordecone) depend not merely on its metabolic activation, but also to a substantial degree on the livers hepatocellular regenerative capacity (e.g., Mehendale 1990, 1991, 1992). For example, the auto protection conferred by a low nontoxic dose of carbon tetrachloride against the toxic effects of a subsequent high dose seem not to be completely accounted for by mere destruction of

cytochrome P-450 activation capacity, but appear also to involve the early (2–6 hours after pretreatment) stimulation of hepatocellular regeneration (Rao and Mehendale 1991; Thakore and Mehendale 1991). This early, low-dose stimulation, which leads to much greater hepatocellular regenerative activity (DNA-synthesis and mitosis) following the high-dose exposure, and the autoprotection phenomenon are both inhibited by a colchicine-induced mitotic block (Rao and Mehendale 1991, 1993). It has been hypothesized that the low dose of carbon tetrachloride and/or the resulting minimal injury induces hepatocytes into the cell cycle from an arrested G_2 state (Calabrese et al. 1993). Further, partial hepatectomy in rats has been shown to confer resistance to carbon tetrachloride-induced hepatotoxicity, presumably via enhanced regenerative capacity, as hepatic uptake and metabolism of carbon tetrachloride was not significantly altered (Young and Mehendale 1989). The particular sensitivity of gerbils to carbon tetrachloride-induced hepatotoxicity appeared related not only to extensive bioactivation, but also to a sluggish hepatocellular regenerative and tissue repair response, and was mitigated by partial hepatectomy that stimulated this response in the absence of any significant effect on carbon tetrachloride bioactivation or induced lipid peroxidation (Cai and Mehendale 1990, 1991a, 1991b). Finally, in rats, pretreatment with nontoxic levels of chlordecone has been shown to substantially potentiate the hepatotoxicity of low doses of carbon tetrachloride without affecting its hepatic metabolism to a similarly significant degree, whereas phenobarbital pretreatment induced greater bioactivation, but less hepatotoxicity (Mehendale and Klingensmith 1988; Young and Mehendale 1989). This chlordecone potentiation phenomenon has been attributed to its inhibitory effect on the level of hepatocellular regeneration and tissue repair normally induced by low-dose carbon tetrachloride, with death resulting from hepatic failure and hepatic encephalopathy (renal toxicity was not affected) (Kodavanti et al. 1992; Soni and Mehendale 1993). Where chlordecone cannot inhibit this regenerative response, as in cultured rat hepatocytes (Mehendale et al. 1991) or gerbils (Cai and Mehendale 1990), it does not potentiate cellular or hepatic toxicity.

3.5.3 Animal-to-Human Extrapolations

Patterns of toxicity and metabolism of carbon tetrachloride in laboratory animals are very similar in humans and animals. In both, similar effects are observed in the major target organs, the liver and kidney, as well as in the nervous system during acute inhalation exposures. There are some minor species differences in metabolic parameters following exposure to carbon tetrachloride. In evaluating inhalation exposures, Thrall et al. (2000) determined that absorption through the lung is lower in humans than in rats or mice. Benson et al. (2001) reported that the fraction of carbon tetrachloride (equivalents following inhalation of radiolabeled carbon tetrachloride) partitioning to the liver after inhalation exposure had the following species ranking: mouse>hamster>rat. Rats eliminated less radioactivity associated with

metabolism and more associated with the parent compound in exhaled air than mice or hamsters. Thrall et al. (2000) determined that humans at low inhalation concentrations metabolized less of the dose than rats, and humans would be less sensitive than rats at equivalent exposures; the rate of metabolism was highest in mice, followed by rat, and then humans. In humans, rats, and mice, CYP2E1 is the major enzyme responsible for bioactivation of carbon tetrachloride. Overall, the toxicokinetic data suggest that humans are less sensitive to carbon tetrachloride than laboratory animals. Therefore, risk assessments for carbon tetrachloride based on animal studies are unlikely to underestimate the potential risk to human health.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Colborn and Clement (1992), was also used in 1996 when Congress mandated the Environmental Protection Agency (EPA) to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), which in 1998 completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral

function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

There is no reported direct effect of carbon tetrachloride on hormones in humans or animals. Fertility was reduced in an inhalation bioassay in rats, but it is not known whether the the cause was hormonal disruption or a necrotic effect on the gonads (Smyth et al. 1936). Testicular degeneration, possibly resulting from necrosis, was observed in rats exposed by inhalation (Adams et al. 1952; Chapman et al. 1992). Adrenal pheochromocytomas were induced in mice exposed to carbon tetrachloride vapor for 2 years (Japan Bioassay Research Center 1998). It is possible that catecholamine balances were affected in these animals (Landsberg and Young 1998). An oral-route assay in rats did not result in reproductive impairment, which suggests that hormones related to reproduction were not affected (Alumot et al. 1976).

It is possible that the loss of hepatic function caused by carbon tetrachloride could indirectly impair hormone metabolic processes that are regulated by the liver. Functions that could be affected by reduced liver function include inactivation of some hormones (e.g., insulin and glucagon) by proteolysis or deamination, deiodination of thyroxine and triiodothyronine, inactivation of steroid hormones (e.g., glucocorticoids and aldosterone) followed by glucuronidation, metabolism of testosterone to 17-ketosteroids and sulfonation, conversion of estrogens to estriol and estrone followed by conjugation to glucuronic acid or sulfate, and removal of circulating vasoactive substances such as epinephrine and bradykinin (Podolsky and Isselbacher 1998). In humans, chronic liver disease not caused by carbon tetrachloride is known to result in signs of hormonal imbalance such as testicular atrophy (Podolsky and Isselbacher 1998). The development of ascites in chronic liver disease may be facilitated by the elevated levels of epinephrine (Podolsky and Isselbacher 1998).

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and in vitro models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6 Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their

alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

One epidemiological study reported associations between maternal exposure to carbon tetrachloride in drinking water and adverse developmental outcomes (low full-term birth weight, small for gestational age, and neural tube defects) in humans (Bove et al. 1992a, 1992b, 1995). Associations between exposure and incidences of central nervous system defects, cleft-lip or cleft-palate, or heart conotruncal defects were not statistically significant (Bove et al. 1992a, 1992b, 1995; Croen et al. 1997). No teratogenic effects were observed in rats exposed to carbon tetrachloride either by inhalation (Gilman 1971; Schwetz et al. 1974) or ingestion (Wilson 1954). Complete litter loss occurred in some rats given oral doses that produced clear maternal toxicity (Narotsky et al. 1997a, 1997b; Wilson 1954). It is not known whether litter loss is the result of toxicity to the fetus or to the placenta, but the critical site of injury is likely related to the abundance of cytochrome proteins that metabolize carbon tetrachloride.

Fetal tissues and the placenta appear to have the capacity for bioactivating carbon tetrachloride, although the levels of cytochrome enzymes are lower than in neonates or adults (EPA 2001). Total fetal liver CYP content is a relatively constant 30% of the adult level from the end of the first trimester of gestation up to 1 year of age (EPA 2001). mRNA for CYP2E1 has been detected in human first-trimester placentas (Hakkola et al. 1996). Low levels of CYP2E1 protein have been detected in human fetal brain as early as gestational day 46, substantially increasing around day 50 (Boutelet-Bochan et al. 1997; Brzezinski et al. 1999). In the fetal liver, CYP2E1 protein was not detectable at 10 weeks of gestation, but was present at 16 weeks (Carpenter et al. 1996). Therefore, it would appear that there is a period early in gestation during which the fetal brain might be more vulnerable than the liver to the effects of carbon tetrachloride. However, no developmental studies are available that specifically examined neurological or neurobehavioral effects of exposure to carbon tetrachloride during gestation. Additionally, there is some evidence that maternal alcohol consumption induces placental CYP2E1 in humans (Rasheed et al. 1997b). If maternal alcohol exposure also increases levels of CYP2E1 in fetal tissues, the likelihood of fetal injury from exposure to carbon tetrachloride would be increased. Induction of fetal hepatic CYP2E1 by maternal ethanol consumption has been confirmed in rats (Carpenter et al. 1997). Transcription of the CYP2E1 gene in human placenta and fetal lung and kidney is regulated in part by hypermethylation of dinucleotide CG residues within the promoter (Viera et al. 1998).

Hepatic levels of CYP2E1 mRNA increase significantly during the first 24 hours after birth, largely resulting from demethylation that allows transcription to proceed (Viera et al. 1996). Major

accumulations of CYP2E1 occur between 1 and 3 months of age and values comparable to those of adults are achieved sometime between 1 and 10 years of age (EPA 2001; Viera et al. 1996). Thus, children exposed to carbon tetrachloride would be expected to experience similar effects as in adults.

Fisher et al. (1997) have calculated that maternal exposure to carbon tetrachloride is likely to result in its transfer to breast milk, which would be a possible means of exposure for nursing infants.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to carbon tetrachloride are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung

capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by carbon tetrachloride are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10 "Populations That Are Unusually Susceptible".

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Carbon Tetrachloride

Measurement of parent carbon tetrachloride and its metabolites in expired air has been the most convenient way to determine exposure. Levels of 9.5 ppm carbon tetrachloride were detected in expired air of one worker who had been exposed to carbon tetrachloride vapors for several minutes (Stewart et al. 1965). In another case, expired air levels were over 2,000 ppm in a person exposed by ingestion to a pint of carbon tetrachloride mixed with methanol (Stewart et al. 1963). Levels fell below 2 ppm after 16 days. Depending on dose and length and route of exposure, the half-life of carbon tetrachloride in expired air initially appears to range from 1 to several hours, later lengthening to 40–>85 hours. Measurement of carbon tetrachloride in blood has also been used as an indicator of exposure.

Covalent adducts between reactive carbon tetrachloride metabolites (trichloromethyl radical) and cellular protein, lipids, and nucleic acids are known to occur. Although measurements of such adducts may provide data on past exposure, the method's overall usefulness in assessing exposure in the general population is severely limited since it requires the use of radiolabeled carbon tetrachloride. Further, metabolite compounds and their adducts may originate in ways other than from carbon tetrachloride, or they may undergo reduction and thus require some reoxidation procedure prior to being detectable by *in vivo* spin trapping techniques (Sentjure and Mason 1992).

3.8.2 Biomarkers Used to Characterize Effects Caused by Carbon Tetrachloride

As discussed in Section 3.2, the effects that are most often observed in humans exposed to carbon tetrachloride are liver and kidney injury and central nervous system depression. Exposure levels leading to these effects in humans are not well-defined. The threshold for central nervous system effects

following exposures of 8 hours or more is probably in the range of 20–50 ppm (Elkins 1942; Heimann and Ford 1941; Kazantzis and Bomford 1960). On the other hand, kidney and liver effects can occur following exposure (15 minutes to 3 hours) to vapor concentrations of 200 and 250 ppm, respectively (Barnes and Jones 1967; Norwood et al. 1950). These exposures correspond to an absorbed dose of approximately 100–200 mg/kg.

Detection of liver injury has commonly been associated with alterations in serum levels of certain hepatic enzymes and proteins. Elevation in bilirubin levels following exposure (Barnes and Jones 1967) has been detected in humans, as have decreased serum levels of secreted liver proteins (e.g., albumin and fibrinogen) (Ashe and Sailer 1942; McGuire 1932; New et al. 1962; Norwood et al. 1950; Straus 1954). Elevations in serum levels of enzymes (alkaline phosphatase and gamma-glutamyltransferase) released from damaged hepatocytes have been reported in occupational exposures above 1 ppm lasting months to years (Tomenson et al. 1995). Similar enzyme elevations were observed following acute-, intermediate-, and chronic-duration exposures to carbon tetrachloride in animals (Bruckner et al. 1986; Hayes et al. 1986; Japan Bioassay Research Center 1998; Sakata et al. 1987). Typically, ALT, AST, alkaline phosphatase, and LDH have been monitored, but these are also produced in nonhepatic tissues. Ikemoto et al. (2001) investigated serum levels of several urea-cycle enzymes that are more exclusively found in the liver: liver-type arginase (ARG), ornithine carbamoyltransferase (OCT), and arginosuccinate synthase (AS). After rats were injected with carbon tetrachloride, serum ARG levels were immediately elevated at the first 15-minute timepoint and within 30 minutes, were about 45-fold higher than normal; after 300 minutes, the increase in serum ARG levels had not reached a plateau. All other enzymes (AST, ALT, OCT, and AS) measured had maximally 10-fold increases. The authors propose that ARG is a sensitive biomarker for acute exposure to carbon tetrachloride and attribute its pattern of appearance in serum to the fact that it is a cytosolic enzyme (having only the plasma membrane as a barrier to the extracellular compartment) and to its smaller molecular mass compared to the other enzyme biomarkers.

In the rat, carbon tetrachloride-induced liver cytolysis has been associated with elevated serum activities of glutamate dehydrogenase, sorbitol dehydrogenase, and glucose-6-phosphatase (microsomal glucose-6-phosphatase activity was decreased) (Brondeau et al. 1991), while serum procollagen III peptide was demonstrated to be a valuable indicator of liver fibrogenesis, and serum prolidase was shown to be a limited signal of accelerated liver collagen metabolism (Jiang et al. 1992). Serum immunoassay for the 7S fragment of type IV collagen may be an even more sensitive indicator of hepatic fibrosis in man (Ala-Kokko et al. 1992). Another sensitive (but nonspecific) indicator of liver injury is the serum levels of individual bile acids (Bai et al. 1992). Lipid peroxidation, increased erythrocyte membrane

cholesterol/phospholipid ratio, and decreased erythrocyte ATPase activity were all associated with the onset of carbon tetrachloride-induced liver cirrhosis (Mourelle and Franco 1991). Also, lipid peroxidation accompanying carbon tetrachloride-induced hepatotoxicity has been monitored by quantitating hepatic levels of hydroperoxy- and hydroxy-eicosatetraenoic acids (Guido et al. 1993).

Renal injury has been associated with acute exposure of humans to carbon tetrachloride. Impaired renal function as evidenced by oliguria and anuria have been reported (Barnes and Jones 1967; Norwood et al. 1950). Proteinuria, hemoglobinuria, and glycosuria have also been reported in other cases involving acute exposure of humans to the compound (Guild et al. 1958; New et al. 1962; Smetana 1939; Umiker and Pearce 1953). Although acute renal failure induced in rats by carbon tetrachloride apparently did not involve activation of the circulating active renin-angiotensin system, increased prorenin levels were associated with decreased renal function (Cruz et al. 1993). These renal effects can occur following exposure to chemicals other than carbon tetrachloride.

Neurotoxicity, as evidenced by central nervous system depression, has been associated with acute exposure to carbon tetrachloride in humans. Clinical signs and symptoms that may be monitored include headache, dizziness, fatigue, and coma (Cohen 1957; Stevens and Forster 1953; Stewart et al. 1961). Impaired visual functions have also been observed (Johnstone 1948; Smyth et al. 1936; Wirtschafter 1933). It should be noted that central nervous system effects disappear rapidly as carbon tetrachloride is eliminated from the body. Therefore, they will be detectable for only relatively short periods after exposure. The neural effects are not specific to carbon tetrachloride exposure and may occur following exposure to other chemicals.

Lipid peroxidation products appearing in urine following exposure to carbon tetrachloride offer the possibility of noninvasive monitoring for hepatic damage (de Zwart et al. 1998). As measured by gas chromatography, the urinary levels in rats of the following lipid peroxidation products showed statistically significant increases over normal values within 12 hours of an intraperitoneal injection with 0.5 or 1.0 mL/kg carbon tetrachloride: formaldehyde, acetaldehyde, propanal, butanal, pentanal, hexanal, and malondialdehyde (MDA). The 0.25 mL/kg dose elicited significant increases only in acetaldehyde. The level of MDA returned to normal after 48 hours, at which time the levels of the other chemicals remained elevated. The same study found that neither coproporphyrin III nor 8-hydroxy-2'-deoxy-guanosine were suitable urinary biomarkers for exposure to carbon tetrachloride.

Metabonomics is a new technology combining high resolution nuclear magnetic resonance (NMR) and pattern recognition technology that is starting to be applied to the evaluation of *in vivo* toxicology. Robertson et al. (2000) treated rats with single intraperitoneal or oral doses of carbon tetrachloride and evaluated the changes in NMR spectra of urine as displayed by principal component analysis (PCA), a statistical method that reduces multidimensional data to a two- or three-dimensional pattern. The PCA pattern was most altered compared to the pretreatment state on the first and second days after treatment, but had returned to normal within 10 days. PCA patterns were detectable in rats treated with 0.5 mg/kg, but not in rats treated with 0.1 mg/kg.

Additional information concerning biomarkers for effects on the immune, renal, and hepatic systems can be found in the CDC/ATSDR Subcommittee Report on Biological Indicators of Organ Damage (CDC/ATSDR 1990), and on the neurological system in the Office of Technology Assessment Report on Identifying and Controlling Poisons of the Nervous System (OTA 1990).

3.9 INTERACTIONS WITH OTHER CHEMICALS

There is substantial evidence that the toxicity of carbon tetrachloride is dramatically increased by alcohols, ketones and a variety of other chemicals. Many of these might be found at hazardous waste sites also containing carbon tetrachloride. Although the precise mechanisms for this marked potentiation are not always known, it is likely that most potentiators act, at least in part, by increasing the metabolic activation of carbon tetrachloride to its toxic intermediates and metabolites, thus increasing the induced injury. Other agents may affect the toxic outcome by altering cellular regenerative and tissue repair capacities. The extent to which either or both of these mechanisms are involved in the interaction will substantially affect the relationships among induced injury, duration of toxic damage, and animal survival. Interactions with agents enhancing lipid peroxidation would be expected to increase the severity of cell injury due to increased permeability of cell membranes.

Ethanol. Alcohol (ethanol) ingestion has often been associated with potentiation of carbon tetrachloride-induced hepatic and renal injury in humans (Manno et al. 1996). In two cases in which men cleaned furniture and draperies with carbon tetrachloride, one man, a heavy drinker, became ill and died, whereas his coworker, a nondrinker, suffered a headache and nausea, but recovered quickly after breathing fresh air (Smetana 1939).. Both men were subjected to the same carbon tetrachloride exposure, as they had been working in the same room for the same amount of time. In 19 cases of acute renal failure due to carbon tetrachloride inhalation or ingestion, 17 of 19 patients had been drinking alcoholic beverages at

about the time of their carbon tetrachloride exposure (New et al. 1962). Many other cases of carbon tetrachloride-induced hepatic and/or renal injury associated with ethanol ingestion have been described in the medical literature (Durden and Chipman 1967; Guild et al. 1958; Jennings 1955; Lamson et al. 1928; Markham 1967; Tracey and Sherlock 1968). These clinical reports establish that occasional or frequent ingestion of alcoholic beverages can increase the danger from exposure to carbon tetrachloride at levels that otherwise do not result in significant toxicity. As ethanol is known to induce microsomal mixed-function oxidase activity in man (Rubin and Lieber 1968), the mechanism of potentiation may involve ethanol-induced enhancement of the metabolic activation of carbon tetrachloride.

Numerous studies in animals confirm that ethanol is a strong potentiator of carbon tetrachloride-induced hepatotoxicity (Ikatsu et al. 1991; Kniepert et al. 1991; Wang et al. 1997a). Ethanol administration 16–18 hours before carbon tetrachloride exposure potentiated hepatotoxicity (Cornish and Adefuin 1966; Towner et al. 1991); however, enhancement was less when ethanol was given 2 hours before carbon tetrachloride (Cornish and Adefuin 1966). This is consistent with the idea that ethanol increases carbon tetrachloride toxicity by inducing the synthesis of one or more enzymes, such as cytochrome P-450 2E1 (Castillo et al. 1992), that are involved in the metabolic activation of carbon tetrachloride; or by acting as a competitive inhibitor of carbon tetrachloride metabolism during concurrent exposure. Thus, the precise timing of exposure to each agent is likely to critically influence the observed effects. For example, a single dose of ethanol 18 hours prior to intraperitoneal administration of 1,275 mg/kg carbon tetrachloride in rats did not increase either trichloromethyl free-radical adducts or p-nitrophenol hydroxylase activity (Reinke et al. 1992). Threshold levels also appear involved, as 14 days of 0.05–0.5 mL/kg/day ethanol did not result in a statistically significant increase in any effects of a subtoxic 20 mg/kg/day dose of carbon tetrachloride (Berman et al. 1992). Ethanol exposure intensified carbon tetrachloride toxicity in pregnant rats and caused decreased postnatal survival of offspring (Gilman 1971). For the most part, these studies involved short-term exposures to ethanol. Inhalation studies involving longer-term pretreatment exposures to ethanol (5–10 weeks) prior to carbon tetrachloride exposure raised the possibility of increased susceptibility to chronic liver injury at low doses of carbon tetrachloride that have not been shown to cause significant liver damage (Hall et al. 1990). On the other hand, when ethanol pretreatments increased in duration (30 or 52 weeks), there was a decrease in ethanol potentiation of carbon tetrachloride toxicity (Kniepert et al. 1990). Factors contributing to this diminished potentiation were not determined. It has also been reported that despite substantial potentiation of carbon tetrachloride-induced hepatotoxicity in ethanol pretreated rats, no increase in lethality was observed (Ray and Mehendale 1990). The authors speculated that this result occurred due to the treatment's concomitant stimulation of hepatic regenerative capacity—to a degree sufficient to overcome the induced injury. In

addition to enhanced hepatotoxicity pretreatments with ethanol have been reported to enhance certain immunosuppressive effects of carbon tetrachloride (Kaminski et al. 1990).

Other Alcohols and Ketones. Secondary alcohols can also potentiate carbon tetrachloride hepatorenal toxicity in humans. Eighteen workers in an isopropyl alcohol packaging plant became ill after inhalation of carbon tetrachloride (Folland et al. 1976). Four of these people were hospitalized; one with liver injury, one with kidney damage, and the other two with both kidney and liver injury. Air samples taken at the plant during a subsequent investigation revealed relatively high concentrations of isopropanol and acetone, and these were thought to play a major role in potentiation of toxicity. Potentiation of carbon tetrachloride hepatoxicity in mice by isopropanol far exceeded that caused by an equal dose of ethanol, though both exerted their maximum effect when given 18 hours before carbon tetrachloride (Traiger and Plaa 1971). In rats, isopropanol potentiated hepatic injury caused by carbon tetrachloride, but lethality was not increased because of the augmentation of hepatic tissue repair mechanisms (Rao et al. 1996). Methanol co-treatment in rats potentiated the hepatotoxicity of carbon tetrachloride by inducing CYP2E1 in rat liver (Allis et al. 1996). Methanol was found to be markedly less effective on an equimolar basis than either isopropanol or tertiary-butanol in enhancing carbon tetrachloride-induced hepatotoxicity in rats (Harris and Anders 1980). These differences likely reflect the substantially longer half-lives of the secondary and tertiary compounds (relative to their primary congeners), which makes them more potent and persistent inducers of cytochrome P-450 activities. Methanol, ethanol, isopropanol, or decanol in combination with carbon tetrachloride caused massive liver damage, but failed to increase carbon tetrachloride induced lethality. On the other hand, tert-butanol, pentanol, hexanol, and octanol not only potentiated liver damage when administered prior to carbon tetrachloride, but also significantly increased the lethal effects of carbon tetrachloride (Ray and Mehendale 1990). Thus, potentiated hepatotoxicity, as measured by various endpoints, may not be a very reliable predictor of the eventual survival outcome. Other experiments in rats demonstrated that both isopropanol and acetone (the major metabolite of isopropanol) are apparently responsible for the marked enhancement of carbon tetrachloride hepatotoxicity (Plaa and Traiger 1972). Similarly, the metabolism of 2-butanol to 2-butanone contributed to the marked ability of this alcohol to potentiate carbon tetrachloride hepatotoxicity in rats (Traiger and Bruckner 1976).

Investigations in rats indicate that ketosis, caused either by diabetes or administration of ketones, can potentiate carbon tetrachloride hepatotoxicity. Pre-treatment with methyl isobutyl ketone, acetone, or metyl ethyl ketone increased hepatotoxicity in rats treated with a single dose of carbon tetrachloride, essentially reducing the ED_{50} for carbon tetrachloride by 80, 73, or 89%, respectively (Raymond and Plaa

1995). Hepatotoxicity (fibrosis and cirrhosis) and nephrotoxicity were increased in rats exposed to both acetone and carbon tetrachloride (Charbonneau et al. 1986). Carbon tetrachloride hepatotoxicity increased in diabetic rats (Hanasono et al. 1975), while 1,3-butanediol induced ketosis and potentiated carbon tetrachloride hepatoxicity (Pilon et al. 1986). In both studies, ketosis was a better index for prediction of liver injury than glycemic status. Interestingly, the same specific form of cytochrome P-450 was reported to be induced in rats by chronic ethanol administration (Joly et al. 1977) and by diabetes (Past and Cook 1982). The bulk of available evidence suggests that elevated levels of ketone bodies induce the enzyme system responsible for biotransformation of carbon tetrachloride to its reactive metabolites (Pilon et al. 1986). Methyl isobutyl ketone significantly increased total levels of cytochrome P-450 in rat liver microsomes (Raymond and Plaa 1995).

Phenobarbital, Metamphetamine, DDT, PBB, Chlordecone. Phenobarbital (PB) has been shown to produce a marked increase in carbon tetrachloride hepatotoxicity in rats and it is widely used to provide experimental animal models of carbon tetrachloride-induced cirrhosis (Abraham et al. 1999; Cornish et al. 1973; Garner and McLean 1969; Hocher et al. 1996; Sundari et al. 1997). This is not surprising, in that cytochrome P-450 PB-B (CYP2B1), the isozyme that can be induced at least 50-fold in rats by PB, participates in the metabolic activation of carbon tetrachloride (Vittozzi and Nastainczyk 1987). Lethal effects of carbon tetrachloride are not potentiated by even large doses of phenobarbital in spite of increased liver injury. Thus, as with the alcohols, manifestations of bioactivation capacity or hepatic injury do not appear to reliably predict the eventual survival outcome. The mechanism underlying this phenomenon appears to be the stimulation of hepatic regeneration and tissue repair. Although the early phase of hepatic regeneration was postponed from 6 to 24 hours, it was greatly increased at 24 and 48 hours. Therefore, in spite of remarkably increased liver injury, the animals are able to overcome injury and survive the potentiated liver toxicity (Kodavanti et al. 1992; Mehendale 1990, 1991, 1992). Some data suggest that the PB-induced P-450 isozyme(s) are more rapidly inactivated by carbon tetrachloride, and that PB pretreatment may alter the target lipids and/or the initiating metabolites involved in lipid peroxidation and diene conjugate formation (Moody 1992). DDT increased the sensitivity of rats to carbon tetrachloride poisoning (McLean and McLean 1966), and mice fed 100 ppm polybrominated biphenyls (PBBs) or 200 ppm polychlorinated biphenyls (PCBs) in their diet for 28 days experienced increased carbon tetrachloride hepatotoxicity (Kluwe et al. 1979). Potentiation of renal dysfunction was also found in the PBB-pretreated mice. All of these compounds are broad-spectrum mixed-function oxidase (MFO) inducers.

Concurrent treatment with methamphetamine at doses between 5 and 15 mg/kg increased hepatotoxicity in rats treated with carbon tetrachloride (Roberts et al. 1994). No potentiation occurred when metamphetamine was administered several hours before or after administration of carbon tetrachloride.

Low dietary doses (10 ppm) of the insecticides chlordecone or mirex (a structural analog of chlordecone) have been demonstrated to potentiate carbon tetrachloride hepatotoxicity. Chlordecone greatly enhanced the hepatotoxicity of carbon tetrachloride in rats, producing cholestasis as well as hepatocellular damage (Curtis et al. 1979). The investigators conclude that there is the likelihood of severe liver damage resulting from interaction of carbon tetrachloride and chlordecone at exposure levels which may independently be nontoxic. Chlordecone has been reported not to potentiate the renal toxicity in rats (Kodavanti et al. 1992) or neurotoxicity in gerbils (Desaiah et al. 1991) of carbon tetrachloride, so its enhancing effects may be liver-specific. Chlordecone potentiation of carbon tetrachloride hepatotoxicity and lethality appears due to incapacitation of hepatocytes to regenerate and initiate the early phase of tissue repair. The authors also suggest that this is due to a precipitous depletion of cellular ATP that results from increased intracellular accumulation of Ca^{2+}, which in turn leads to a depletion of glycogen (Bell and Mehendale 1987; Mehendale 1990, 1991, 1992; Soni and Mehendale 1993). Mirex pretreatment of carbon tetrachloride-dosed rats was found not to produce cholestasis, but to produce a relatively modest increase in carbon tetrachloride hepatotoxicity (Bell and Mehendale 1985). Pretreatment of carbon tetrachloride-dosed rats with both mirex and chlordecone did not increase hepatotoxicity above that seen with chlordecone alone, indicating that chlordecone influenced susceptibility to carbon tetrachloride in a way independent of that of mirex. As proposed for phenobarbital, the mechanism underlying only limited and low-grade potentiation of carbon tetrachloride by mirex may involve a stimulation of hepatic regeneration and tissue repair that offsets cytochrome P-450 induction (Mehendale 1990, 1991, 1992). A single oral dose of chlordecone enhanced the oxidative metabolism of carbon tetrachloride in rats, but to a lesser degree than PB, which was in inverse relationship to these agents' effects on potentiation of the lethal and hepatotoxic effects of carbon tetrachloride (Mehendale and Klingensmith 1988). The investigators suggested the involvement as of yet unidentified factors, in addition to the modest enhancement of carbon tetrachloride metabolism, in chlordecone's unusually strong potentiating capacity. As discussed above, subsequent studies have suggested that chlordecone potentiates carbon tetrachloride-induced hepatotoxicity by depleting cellular energy stores, and consequently by inhibiting hepatocellular regeneration and liver tissue repair (e.g., Kodavanti et al. 1992; Mehendale 1991, 1992; Soni and Mehendale 1993).

Haloalkanes. Certain haloalkanes and haloalkane-containing mixtures have been demonstrated to potentiate carbon tetrachloride hepatotoxicity. Pretreatment of rats with trichloroethylene (TCE) enhanced carbon tetrachloride-induced hepatotoxicity, and a mixture of nontoxic doses of TCE and carbon tetrachloride elicited moderate to severe liver injury (Pessayre et al. 1982). The researchers believed that the interaction was mediated by TCE itself rather than its metabolites. TCE can also potentiate hepatic damage produced by low (10 ppm) concentrations of carbon tetrachloride in ethanol pretreated rats (Ikatsu and Nakajima 1992). Acetone was a more potent potentiator of carbon tetrachloride hepatotoxicity than was TCE, and acetone pretreatment also enhanced the hepatotoxic response of rats to a TCE-carbon tetrachloride mixture (Charbonneau et al. 1986). The potentiating action of acetone may involve not only increased metabolic activation of TCE and/or carbon tetrachloride, but also possible alteration of the integrity of organelle membranes. Carbon tetrachloride-induced liver necrosis and lipid peroxidation in the rat have been reported to be potentiated by 1,2-dichloroethane in an interaction that does not involve depletion of reduced liver glutathione, and that is prevented by vitamin E (Aragno et al. 1992; Danni et al. 1992). Dichloromethane potentiated the hepatotoxicity of carbon tetrachloride in rats by increasing the covalent binding of carbon tetrachloride metabolites to hepatic microsomal lipids (Kim 1997). Several anesthetics (isoflurane, enflurane, halothane, and sevoflurane) enhanced the dechlorination of carbon tetrachloride by guinea pig microsomes by stimulating the reduction of cytochrome P-450 (Fujii 1996; Fujii et al. 1996).

Nicotine. Treatment of rats for 10 days with nicotine in drinking water increased liver histopathology (fatty change, necrosis, and dark-cell change) caused by an injection of carbon tetrachloride (Yuen et al. 1995). It was proposed that the increased hepatotoxicity might have resulted from a synergistic effect of the lipid peroxidation induced by both agents. Pregnant rats showed less severe effects than nonpregnant rats, possibly because of the differential hormonal status or differential expression of CYP450 enzymes.

Carbon Disulfide and Other Alkyl Sulfides. Just as chemicals that serve to stimulate the metabolism of carbon tetrachloride lead to increased toxicity, chemicals that impair carbon tetrachloride metabolism lead to decreased toxicity. Rats dosed with carbon disulfide together with carbon tetrachloride displayed effects on the liver that resembled those due to carbon disulfide alone, rather than those caused by carbon tetrachloride alone (Seawright et al. 1980). This was judged to be due to destruction of the hepatic P-450 metabolizing system by carbon disulfide, such that activation of carbon tetrachloride was much reduced. Similar results have been reported in workers exposed to "80/20" (a mixture of carbon tetrachloride and carbon disulfide used to fumigate grain) (Peters et al. 1987). The neurological effects observed in these

individuals resembled those caused by carbon disulfide alone, and there was no evidence of hepatotoxic effects characteristic of carbon tetrachloride exposure.

Other sulfides administered as pretreatments had different effects on carbon tetrachloride hepatotoxicity as measured by plasma ALT levels (Kim et al. 1996). The increase in plasma ALT levels induced by carbon tetrachloride was blocked by pretreatment with allyl sulfide or allyl disulfide and increased by pretreatment with propyl disulfide and butyl sulfide.

Dietary Status. Because carbon tetrachloride causes injury through oxidative pathways, depletion of cellular antioxidants such as glutathione, vitamin E and methionine tend to increase the toxicity of carbon tetrachloride. For example, feeding rats a diet low in vitamin E, selenium (a required cofactor for glutathione reductase), and methionine led to increased lipid peroxidation, while feeding a diet supplemented with one or more of these antioxidants tended to decrease lipid peroxidation (Hafeman and Hoekstra 1977) and oxidative liver damage (Parola et al. 1992). Similar results have been obtained by Taylor and Tappel (1976) and Sagai and Tappel (1978). In mice, retinoic acid or retinol inhibited the carbon tetrachloride-induced increase in serum alanine transaminase activity and liver histopathology, suggesting a protective effect of vitamin A in mice (Kohno et al. 1992; Rosengren et al. 1995). However, pretreatment with retinol increased hepatocyte injury in rats exposed to carbon tetrachloride (Badger et al. 1996; ElSisi et al. 1993a, 1993b).

Food deprivation has also been shown to have a substantial effect on carbon tetrachloride hepatotoxicity. A 24-hour fast significantly depressed hepatic glutathione (GSH) levels and enhanced carbon tetrachloride hepatotoxicity in rats (Harris and Anders 1980), and promoted lipid peroxidation as measured by malondialdehyde formation (Ikatsu et al. 1991). Diurnal decreases in hepatic GSH levels were found to coincide with periods of maximal susceptibility to carbon tetrachloride hepatotoxicity (Bruckner et al. 1984; Harris and Anders 1980). Even though the role of GSH in carbon tetrachloride cytotoxicity is poorly understood, it appears that more than GSH depletion is involved in fasting-induced enhancement of carbon tetrachloride hepatotoxicity. A 1-day fast stimulates the capacity of liver microsomes from male and female rats to metabolize carbon tetrachloride, although fasting did not produce a significant increase in hepatic microsomal protein or cytochrome P-450 levels (Nakajima and Sato 1979). Thus, short-term food deprivation may enhance the biotransformation of carbon tetrachloride to cytotoxic metabolites. It should be recognized that food deprivation or consumption of a protein-free diet for several days diminishes MFO activity and makes rats more resistant to carbon tetrachloride (McLean and McLean 1966; Seawright and McLean 1967). Food restriction (25 or 50% lower caloric

than control intake) for 30 days prior to administration of carbon tetrachloride reduced the magnitude of blood lipid peroxidation and depressed increases in serum enzymes in carbon-tetrachloride-treated rats. (Ramkumar et al. 2003).

Metals. Pre-exposure to single doses of various metals (hexavalent chromium, mercuric chloride or silver) had no synergistic effect on lipid peroxidation in rats treated with carbon tetrachloride (Rungby and Ernst 1992). Rats fed a low-copper diet were reported to be more sensitive to hepatic plasma membrane injury 24 hours following an intraperitoneal injection of carbon tetrachloride, possibly due to reduced Cu-Zn superoxide dismutase activities (DiSilvestro and Medeiros 1992). Rats fed a diet mildly deficient in zinc showed elevated levels of hepatocyte injury, as assessed by serum sorbitol dehydrogenase activity (DiSilvestro and Carlson 1994). In rats injected with lead nitrate and then carbon tetrachloride, hepatoxicity, as measured by serum ALT and AST, was lower than in rats injected with carbon tetrachloride alone (Calabrese et al. 1995); the authors attributed this effect to the ability of lead to inhibit cytochrome P-450.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to carbon tetrachloride than will most persons exposed to the same level of carbon tetrachloride in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of carbon tetrachloride, or compromised function of organs affected by carbon tetrachloride. Populations who are at greater risk due to their unusually high exposure to carbon tetrachloride are discussed in Section 6.7, Populations With Potentially High Exposures.

Section 3.9 discusses several types of compounds that can exacerbate the toxicity of carbon tetrachloride. Individuals exposed to these compounds may, therefore, be more sensitive to carbon tetrachloride exposure. As noted above, persons who are moderate to heavy drinkers are at significantly increased risk of liver and/or kidney injury following ingestion or inhalation of carbon tetrachloride (Manno et al. 1996). Occupational exposure to isopropanol has also been reported to markedly potentiate the hepatic or renal toxicity of carbon tetrachloride in men and women (Folland et al. 1976). This report and numerous animal studies indicate that primary, secondary, and tertiary alcohols, as well as their ketone analogues, can substantially enhance the toxic potency of carbon tetrachloride. Substantial exposures to alcohols and

ketones may occur in occupational settings or in certain instances in the use of household products containing these chemicals.

Drugs and other chemicals that significantly induce microsomal MFO activity can significantly increase the toxicity of carbon tetrachloride by enhancing its biotransformation to reactive, cytotoxic metabolites. A number of drugs such as phenobarbital, pentobarbital, and phenylbutazone are MFO inducers in animals and humans. Thus, individuals taking such medications may be at substantially greater risk of carbon tetrachloride toxicity. Other unusually susceptible individuals are those who have had significant exposures to insecticides such as DDT, chlordecone, or mirex, or to industrial chemicals such as PCBs or PBBs. All of these chemicals are potent MFO inducers and have been shown to markedly potentiate the hepatotoxicity of carbon tetrachloride in animals. Exposures to these chemicals can occur in industrial and agricultural settings, as well as in the general population via environmental media (i.e., contaminated water, food, air, and soil). Other widely used chemicals such as TCE have been found to enhance carbon tetrachloride toxicity in animals. Thus, persons with substantial exposure to TCE and other haloalkanes may be at greater risk of carbon tetrachloride toxicity.

Nutritional status can also influence the toxic potency of carbon tetrachloride. Animal studies have clearly demonstrated that brief fasting or consumption of diets low in antioxidants (vitamin E, selenium, methionine) can lead to increased carbon tetrachloride hepatotoxicity. The same may be true for humans, although this is not known for certain. Another aspect of nutritional status affecting carbon tetrachloride toxicity is hepatic energy status. Hepatic ATP levels might influence the ultimate outcome of toxicity (low levels may inhibit recovery mechanisms).

A variety of conditions may predispose certain segments of the population to carbon tetrachloride toxicity. Persons with alcoholic cirrhosis, or other liver diseases that have significantly diminished the functional reserve of the liver, have a reduced capacity to tolerate carbon tetrachloride-induced hepatotoxicity. The same is true for carbon tetrachloride-induced nephrotoxicity in people with significant renal dysfunction from other causes. Diabetics may be particularly susceptible to carbon tetrachloride poisoning, in light of animal studies that indicate elevated levels of ketone bodies induce the MFO system, which converts carbon tetrachloride to reactive, cytotoxic metabolites. Individuals with genetically-determined high MFO activity may be more susceptible to carbon tetrachloride toxicity, as may be persons with habits (e.g., smoking, consumption of smoked meats) that can produce increased MFO activity.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to carbon tetrachloride. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to carbon tetrachloride. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to carbon tetrachloride:

Ellenhorn MJ. 1997. Ellenhorn's medical toxicology: diagnosis and treatment of human poisoning. 2nd ed. New York, NY: Elsevier, 1422-1429.

Shih RD. 1998. Hydrocarbons. In: Goldfrank LR, Flomenbaum NE, Lewin NA, et al., eds Goldfrank's toxicologic emergencies. 6th ed. Stamford, CT: Appleton & Lange, 1383-1398.

3.11.1 Reducing Peak Absorption Following Exposure

Human exposure to carbon tetrachloride may occur by inhalation, ingestion, or dermal contact. Inhalation or oral exposure to carbon tetrachloride may cause hepatic, renal, and neurological effects. There is evidence, though limited, that dermal contact causes a similar pattern of effects.

If carbon tetrachloride has been inhaled, movement to fresh air is recommended. Humidified supplemental oxygen (100%) may be administered as required.

Ingestion of carbon tetrachloride should be considered a toxic emergency in which treatment should begin immediately. Treatment currently involves gastric emptying, either by gastric lavage (with a small bore nasogastric tube) or by induction of vomiting, preferably within minutes of exposure (Shih 1998). The patient needs to have a gag reflex and should not show signs of seizure, lethargy, or coma because of the risk of pneumonitis from pulmonary aspiration. In infants and young children, the induction of vomiting may induce severe fluid loss. Supportive therapy should be followed in all instances of treatment. A cathartic may be administered to speed fecal excretion (Ellenhorn 1997). Administration of activated charcoal is unlikely to be effective (Ellenhorn 1997). Animal studies revealed peak blood levels of carbon tetrachloride within 3–6 minutes after oral exposure when carbon tetrachloride was ingested undiluted or in aqueous vehicles by fasted rats (Kim et al. 1990a). Chemicals that induce P-450, such as ethanol and phenobarbital, should not be given. The administration of epinephrine is avoided, due to the possibility of inducing ventricular arrhythmias. In order to minimize absorption through the skin, all

contaminated clothing should be removed and the skin should be washed with soap and water. In cases where the compound has been splashed into the eyes, irrigation with copious amounts of tepid water for 15 minutes has been recommended. Medical treatment is required if irritation, pain, swelling, lacrimation, or photophobia persist.

3.11.2 Reducing Body Burden

Hemodialysis may be employed in order to lower plasma carbon tetrachloride at the onset of renal failure (Ellenhorn 1997). Although this method is not very effective in removing lipophilic compounds from the blood, it is effective in controlling extracellular fluid composition if renal failure occurs (EPA 1989b; Ellenhorn 1997). Because a substantial portion of absorbed carbon tetrachloride is exhaled within the first hour, maintenance of a good tidal volume is recommended; hyperventilation may also be of value (Ellenhorn 1997). Administration of hyperbaric oxygen is an experimental treatment that is also available. Hyperbaric oxygen has been used in treating overdoses of carbon tetrachloride in humans (Larcan and Lorbet 1981; Truss and Killenberg 1982; Zearbaugh et al. 1988). Administration of hyperbaric oxygen following exposure to carbon tetrachloride improved survival from 31 to 96% in rats (Ellenhorn and Barceloux 1988). Hyperbaric oxygen has also been used in treating overdoses of carbon tetrachloride in humans and may correct regional tissue hypoxia and damage, as well as inhibit the P-450-dependent reductive dehalogenation of carbon tetrachloride to the metabolically active trichloromethyl radical in the liver. However, the effectiveness of this method has not been established in humans (Burkhart et al. 1991; Ellenhorn and Barceloux 1988).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Information is limited in humans regarding compounds that interfere with the mechanism of action of carbon tetrachloride. However, there is evidence that liver toxicity associated with exposure to carbon tetrachloride is mediated by reactive metabolites that bind to hepatocytes and initiate lipid peroxidation, thus resulting in loss of cell function. N-acetylcysteine has been suggested to bind the toxic metabolite phosgene and to serve as a precursor for the formation of glutathione (Ellenhorn and Barceloux 1988), and was protective against hepatotoxicity in carbon tetrachloride-exposed rats (Simko et al. 1992). Glutathione, a cellular antioxidant, tends to decrease lipid peroxidation due to carbon tetrachloride ingestion in rats (Arosio et al. 1997; Hafeman and Hoekstra 1977). Agents that foster the maintenance of hepatic reduced glutathione levels have a similar protective effect against carbon tetrachloride: taurine (Vohra and Hui 2001; Waterfield et al. 1993), gamma-glutamylcysteinylethyl ester (Nishida et al. 1998),

and clofibrate (Manautou et al. 1998). Administration of 16,16-dimethyl prostaglandin E2 to block the accumulation of intracellular lipids has also been suggested (Haddad and Winchester 1990; Rush et al. 1986). Administration of fructose 1,6-diphosphate to rats has been shown to decrease carbon tetrachloride liver toxicity by increasing hepatocyte levels of ATP. The ATP thus generated is thought to promote hepatocellular regeneration and tissue repair (Rao and Mehendale 1989). Shertzer and Sainsbury (1991) reported that indole antioxidants 4b,5,9b,10-tetrahydroindeno[1,2-b]indole (THII) and 5,10-dihydroindeno[1,2-b]indole (DHII) inhibited carbon tetrachloride initiation of lipid peroxidation in rat liver microsomes, and protected against hepatotoxicity in rats when administered prior to carbon tetrachloride treatment. The authors suggested that these compounds may be suitable candidates for further development as potential chemoprotective and therapeutic agents for use in human disorders that involve free-radicals. Colchicine and trimethylcolchicinic acid, an analog that does not bind tubulin, prevented decreases in Ca^{2+}-ATP-ase activity, and reduced increases in gamma-glutamyl transpeptidase, alanine aminotransferase, and alkaline phosphatase in hepatocyte plasma membranes in rats treated with carbon tetrachloride (Cedillo et al. 1996; Martinez et al. 1995).

Oxygen supplementation improved ratios of ATP/ADP, inorganic phosphate/ATP, and lactate/pyruvate that had been altered in cirrhotic livers of rats previously treated with carbon tetrachloride (Harvey et al. 2000). These results were consistent with the hypothesis that hepatocyte damage in cirrhotic livers is exacerbated by a reduced oxygen supply and may partly explain the efficacy of hyperbaric oxygen therapy as described in Section 3.11.2).

Compounds that suppress the activity or expression of CYP2E1 have been shown to reduce the hepatic necrosis caused by the bioactivation of carbon tetrachloride. Pretreatment with 100–400 µmol/kg (subcutaneous) oleanolic acid, a triterpenoid compound, reduced heptatoxicity in rats and mice injected with carbon tetrachloride (Liu et al. 1998); the protective effect occurred 12–72 hours after pretreatment and was found to be unrelated to metallothionein levels. In mice, the protective effect of oleanolic acid was associated with inhibition of expression and activity of CYP2E1 (Jeong 1999). Another triterpenoid, alpha-hederin similarly reduced expression of CYP2E1 and hepatic injury in mice treated with carbon tetrachloride (Jeong and Park 1998). Methylenedioxybenzenes such as isosafrole, dihydrosafrole, and benzodioxole, administered 1 hour before carbon tetrachloride, prevented increases in plasma AST and ALT in mice (Zhao and O'Brien 1996). Isosafrole co-treatment also prevented the development of liver necrosis. Safrole was partially hepatoprotective, whereas piperonyl butoxide, eugenol, isoeugenol, sesamol, and curcumin were ineffective. Other similar compounds that prevented increases in plasma AST and ALT in rats included tetrahydro-5-methyl bis[1,3]benzdioxide [4,5-C: 5',6]-azecin-13 (5*H*)-one

(protopine) (Janbaz et al. 1998) and 2-methylaminoethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxy-biphenyl-2-carboxylic acid-2'-carboxylate monohydrochloride (DBB-S) (Oh et al. 2000). A synthetic agent, 2-(allylthio)pyrazine, suppressed constitutive and inducible CYP2E1 expression and also blocked carbon tetrachloride-induced hepatotoxicity in mice (Kim et al. 1997); the compound also elevated hepatic GSH levels.

Tumor necrosis factor alpha (TNF-alpha) has been implicated in the process of hepatocellular injury following exposure to carbon tetrachloride. Co-treatment of rats with the soluble receptor to TNF-alpha reduced hepatocellular necrosis and the elevation in serum enzyme levels caused by carbon tetrachloride (Czaja et al. 1995). Mortality was 16% in the rats co-treated with the soluble receptor and 60% in rats co-treated with IgG.

A number of agents have been shown to reduce the severity of fibrosis induced in animals following intermediate-duration exposure to carbon tetrachloride. A weak but significant reduction in the area of carbon tetrachloride-induced hepatic fibrosis was measured by image analysis in rats co-treated with interferon $alpha_{2a}$ over a period of 9 weeks (Fort et al. 1998). There were concomitant reductions in several biochemical markers of fibrosis (hyaluronate, hydroxyproline, and the mRNAs for procollagen and fibronectin). Administration of interferon-$alpha_{2b}$ also reduced the severity of fibrosis in the kidneys of rats subcutaneously injected with carbon tetrachloride over 7 weeks (Dogukan et al. 2003). Histopathology analysis revealed reductions in necrosis, dilatation and atrophy of renal tubules, hypercellularity of glomeruli, and obliteration of renal capillaries in rats co-treated with interferon compared to placebo-co-treated rats; the level of interstitial fibrosis was also reduced by interferon, although the difference was not statistically significant from the placebo co-treatment group. The kidneys of rats co-treated with interferon had more interstitial inflammation than the rats in the control group or in the placebo-co-treatment group.

Administration of liver growth factor to rats with hepatic cirrhosis following intraperitoneal injections of carbon tetrachloride for 10 weeks significantly improved the structure and function of the liver (Diaz-Gil et al. 1999). Significant decreases were observed in the levels of serum enzymes, the hepatic collagen content, and microscopic findings of fibrosis, necrosis, and inflammatory infiltration of the liver. In addition, hepatic hemodynamic measures were improved in rats treated with liver growth factor compared to cirrhotic rats: reduced portal pressure and portosystemic shunting, reduced ascites, and increased mean arterial pressure and systemic vascular resistance. Implantation of rat fibroblasts genetically modified to express hepatic growth factor into the spleens of syngeneic rats significantly reduced hepatic injury

(serum enzymes, histopathology) resulting from an intraperitoneal injection of carbon tetrachloride (Kaido et al. 1997). Gene therapy using an adenoviral vector bearing cDNA for a nonsecreted form of human urokinase plasminogen activator (Ad-ΔhuPA) reduced hepatic fibrosis in rats that became cirrhotic following treatment with carbon tetrachloride for 6–8 weeks (Salgado et al. 2000). The beneficial effect of enhanced uPA expression was partly attributed to its induction of hepatocyte growth factor.

Treatment of insulin-like growth factor-I (IGF-I) to rats during the last 3 weeks of exposure to carbon tetrachloride/phenobarbitol partially normalized the expression of 8 of 16 genes that were either up- or down-regulated in the cirrhotic liver (Mirpuri et al. 2002). Three of the genes affected by IGF-I are for protease inhibitors; restoration of the expression of these genes would be expected to protect against necrosis. IGF-I treatment also partially restored the expression of growth hormone receptor and the levels of global genomic DNA methylation, which are reduced during the development of cirrhosis (Mirpuri et al. 2002). Evaluation of hepatic effects following IGF-I administration to cirrhotic rats on the same protocol resulted in reductions in lipid peroxidation, fibrosis, and plasma AST and ALT, and increases in mitochondrial transmembrane potential (a measure of mitochondrial membrane integrity) (Castilla-Cortazar et al. 1997).

Several agents have been shown to ameliorate the effect of carbon tetrachloride on hepatic membranes. When co-administered with carbon tetrachloride, betaine, a mitochondrial metabolite of choline, reduced the extent of centrilobular steatosis and minimized the loss of hepatocyte organelle membranes (rough endoplasmic reticulum) in treated rats (Junnila et al. 2000); the effect was attributed to the enhancement of phospholipid synthesis necessary for maintaining the integrity of cell membranes. Hydroxychalcones, which have a 3,4-dihydroxycinnamoyl structure and inhibit lipoxygenases and cyclooxygenases, were potent inhibitors of lipid peroxidation in cultured rat hepatocytes (Sogawa et al. 1994). Polyenylphosphatidyl choline also reduced hepatic fibrosis induced by carbon tetrachloride in rats and accelerated the regression of existing fibrosis (Ma et al. 1996).

As vitamin A (retinol) shows species-specific variations on carbon tetrachloride-related hepatotoxicity, it is not possible to predict whether it would be useful as a therapeutic agent in exposed humans. Pretreatment of male mice with vitamin A for 7 days prior to a single exposure to carbon tetrachloride reduced the elevations in plasma ALT levels as well as the extent of hepatic degeneration (Hooser et al. 1994). Some strain variations were evident in the protective effect of vitamin A, with no hepatocyte damage visible in C3H/He or athymic nude mice and only minimal hepatocyte damage visible near the central vein in Swiss-Webster or Balb/C mice. Conversely, pretreatment with vitamin A increased the

hepatotoxicity (plasma ALT levels) of carbon tetrachloride 10-fold in male and female Sprague-Dawley rats, and male nude and Fischer-344 rats. The underlying basis for the species and strain differences is not known, but the possible involvement of Kupffer cells or polymorphonuclear neutrophils is under investigation. Inder et al. (1999) determined that the effect of vitamin A in Swiss-Webster mice does not involve alteration of the constitutive or inducible expression of CYP2E1.

Avid retention of Na^+ is a feature of liver cirrhosis. Icatibant (HOE 140), an antagonist to the bradykinin B_2 receptor, normalized Na^+ retention and reduced the hyperactivity of the renin-angiotensin-aldosterone system in rats that had become cirrhotic following treatment with carbon tetrachloride (Wirth et al. 1997).

Malnutrition is a common result of cirrhosis. Survival was improved in rats with carbon tetrachloride-induced cirrhosis by the dietary administration of branched-chain amino acids in addition to a casein diet (Kajiwara et al. 1998). Supplementation with branched-chain amino acids significantly preserved plasma albumin concentration and inhibited the occurrence of ascites and hyperammonemia without altering liver histopathology. The authors hypothesize that administration of branched-chain amino acids may suppress muscular protein catabolism and aid in detoxifying excess serum ammonia levels, which are characteristic of cirrhotic patients.

The protective effects of gadolinium a rare earth metal (lanthanide) and glycine against carbon tetrachloride injury operate via inactivation of Kupffer cells, which are hepatic macrophages (Rivera et al. 2001). When either compound was administered to rats with carbon tetrachloride-induced cirrhosis, the livers showed reductions in fibrosis, collagen protein, and transforming growth factor-beta-1 caused by carbon tetrachloride (Rivera et al. 2001). The inactivation of Kuppfer cells by glycine is suspected to be related to the inhibition of calcium signaling via glycine-gated chloride channels (Rivera et al. 2001). Gadolinium chloride also prevented liver injury and increased hepatocyte proliferation (as measured by immunostaining for the hepatocyte proliferating cell nuclear antigen) in rats when administered prior to treatment with carbon tetrachloride (Ishiyama et al. 1995). Gadolinium chloride inhibited CYP2E1 activity in cultured hepatocytes, reducing the loss of plasma membrane integrity caused by carbon tetrachloride (Badger et al. 1997).

Other substances that have been demonstrated to be protective against the toxic effects of carbon tetrachloride in animals include disulfiram (Brady et al. 1991), enprostil, an analog of prostaglandin E_2 (Bang et al. 1992), bosentan, an antagonist to the endothelin receptor (Hocher et al. 1995), the xanthine oxidase inhibitor allopurinol (Dashti et al. 1992), the prolyl 4-hydroxylase inhibitors S 0885 and

HOE 077 (Bickel et al. 1991), pyridoxol L,2-pyrrolidon-5 carboxylate (metadoxine) (Annoni et al. 1992), cyclosporine A (Farghali et al. 1996), the calcium antagonist nifedipine (Cutrin et al. 1992, 1994), alpha-tocopherol and derivatives (Hsiao et al. 2001; Liu et al. 1995), polyamines (Wu et al. 1997), adenosine (Hernandez-Munoz et al. 1992), various phenolic compounds (mostly flavinoids) (Adaramoye and Akinloye 2000; Cholbi et al. 1991), zinc (Camps et al. 1992), and chromium III (but not chromium IV) (Rungby and Ernst 1992; Tezuka et al. 1991a, 1991b). Exercise has been shown to protect subsequently isolated rat hepatocyte from carbon tetrachloride cytotoxicity, probably by affecting cytochrome P-450-2E1 activity, and perhaps also by stimulating intracellular levels of free radical scavengers and antioxidants (Day and Weiner 1991). Food restriction (25 or 50% lower caloric than control intake) for 30 days prior to administration of carbon tetrachloride reduced the magnitude of blood lipid peroxidation and of increases in serum enzymes in carbon-tetrachloride treated rats (Ramkumar et al. 2003).

3.12 ADEQUACY OF THE DATABASE

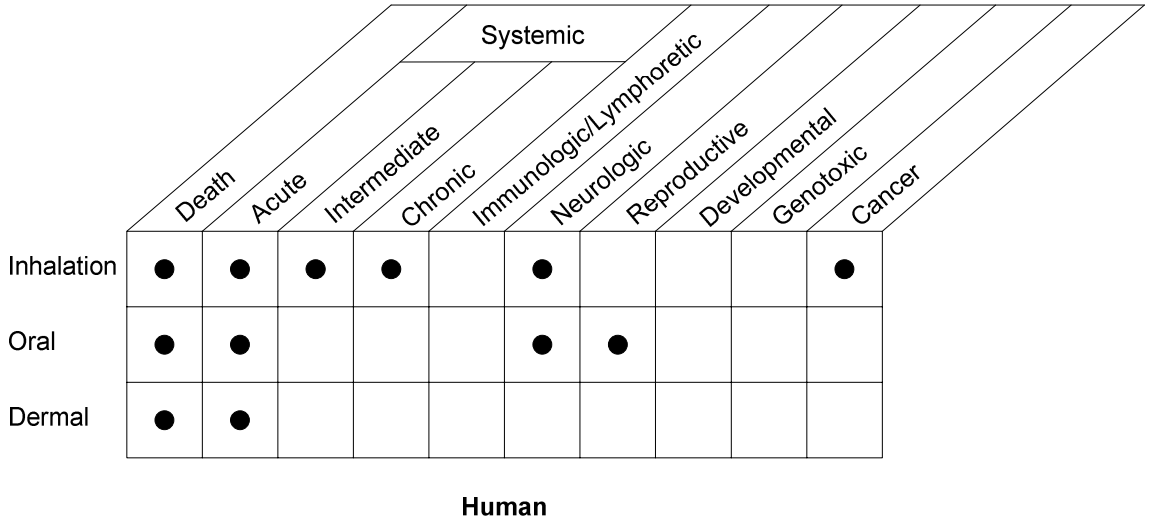
Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of carbon tetrachloride is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of carbon tetrachloride.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

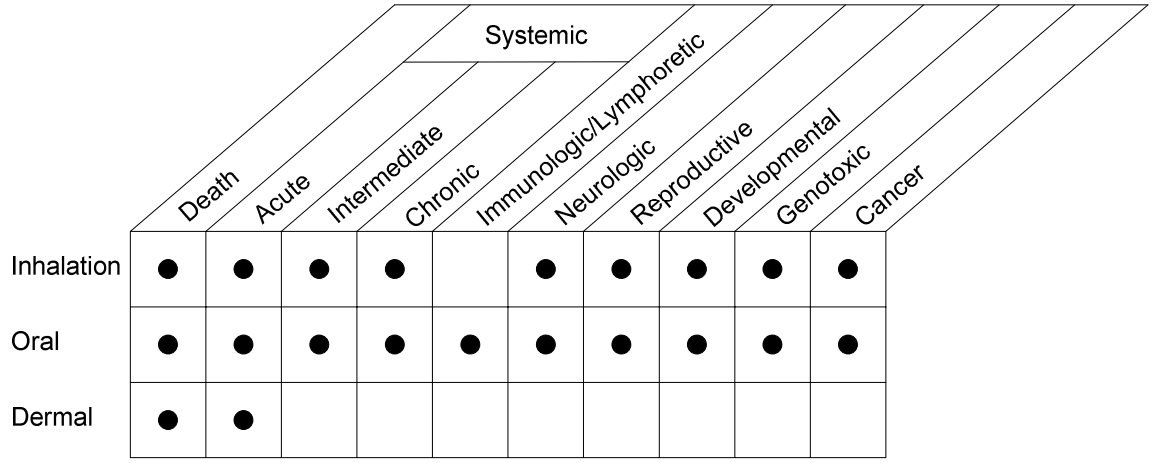
3.12.1 Existing Information on Health Effects of Carbon Tetrachloride

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to carbon tetrachloride are summarized in Figure 3-6. The purpose of this figure is to illustrate the existing information concerning the health effects of carbon tetrachloride. Each dot in the figure indicates that one

Figure 3-6. Existing Information on Health Effects of Carbon Tetrachloride



Human



Animal

● Existing Studies

or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

As shown in Figure 3-6, there is a considerable body of data on the health effects of carbon tetrachloride in humans, especially following acute oral or inhalation exposures. Although many of the available reports lack quantitative information on exposure levels, the data are sufficient to derive approximate values for safe exposure levels. There is limited information on the effects of intermediate or chronic inhalation exposure in the workplace, but there are essentially no data on longer-term oral exposure of humans to carbon tetrachloride. Most toxicity studies have focused on the main systemic effects of obvious clinical significance (hepatotoxicity, renal toxicity, central nervous system depression). There are data on the effects of carbon tetrachloride on the immune system, but there are no reports that establish whether or not developmental, reproductive, genotoxic, or carcinogenic effects occur in humans exposed to carbon tetrachloride.

The toxicity of carbon tetrachloride has been extensively investigated in animals, both by oral and inhalation exposure. While the majority of existing studies in animals have focused on systemic toxicity (hepatic and renal injury), several studies have examined the neurologic, developmental, and reproductive effects of carbon tetrachloride. Effects of carbon tetrachloride on the immune system have been studied following oral, but not after inhalation or dermal exposure. The carcinogenicity of carbon tetrachloride has been studied in animals following inhalation or oral exposure.

3.12.2 Identification of Data Needs

Despite the phase-out of carbon tetrachloride manufacture and use in many areas of the world, its environmental persistence may support the continued practical relevance of many of the data needs identified below.

Acute-Duration Exposure. A large number of studies are available regarding the effects of single exposures to carbon tetrachloride, both in animals and humans. Available data indicate that the central

nervous system, liver, and kidneys are primary target organs for carbon tetrachloride. Many of these studies involved exposure to only one dose level (usually high enough to cause clear effects), and the minimum dose needed to produce the characteristic effects of carbon tetrachloride toxicity is not defined with certainty. Although human studies exist, data were not suitable for derivation of an acute inhalation MRL. An acute inhalation MRL was not derived because calculations based on the most suitable data (exposure of rats at 10 ppm, 7 hours/day for 13 exposures over 17 days in the study by Adams et al. 1952), would result in a value (0.02 ppm) lower than the intermediate-duration inhalation MRL. The intermediate-duration inhalation MRL of 0.03 ppm is expected to be protective for acute-duration inhalation exposures. An acute oral MRL of 0.05 mg/kg/day was also derived based on a LOAEL of 5 mg/kg/day in animals (Smialowicz et al. 1991). Further studies in animals, involving a range of exposure levels and employing sensitive histological and biochemical measurements of injury to liver and kidney, would be helpful in defining the thresholds for acute hepatic and renal toxicity. Studies on the time-course of changes in the most sensitive parameters would be valuable. Most studies are conducted 18–24 hours after exposure. Because carbon tetrachloride is so rapidly absorbed and distributed to target tissues, significant biochemical and histological changes may occur within minutes. These changes may not be evident 18–24 hours later (e.g., Mehendale 1991, 1992). Data for all exposure routes would be valuable, but further information on inhalation and dermal dose-response relationships would be particularly helpful. In addition, dose-response studies of the effects of acute exposures on other tissues and systems (e.g., nervous, immune, reproductive, developmental) would be useful in determining whether other tissues are injured, especially at doses near the thresholds for injury to the liver and kidney. Furthermore, for purposes of enhancing toxicity and risk assessments related to carbon tetrachloride exposure, dose-response studies in species other than rats and gerbils on induced compensatory mechanisms (e.g., hepatocellular regeneration and tissue repair; see, for example, Calabrese et al. 1993; Kodavanti et al. 1992; Mehendale 1990, 1991, 1992; Rao and Mehendale 1991, 1993) might also prove useful.

Intermediate-Duration Exposure. The effects of repeated exposure to carbon tetrachloride have been investigated in a relatively small number of studies. Similar target organs were reported as those for acute-duration exposure. An intermediate inhalation MRL of 0.03 ppm was derived for liver effects in animals based on a NOAEL of 5 ppm in animals (Adams et al. 1952). An oral intermediate MRL of 0.02 mg/kg/day was derived based on an adjusted NOAEL dose of 1 mg/kg/day in animals (Bruckner et al. 1986). There are a number of areas where further studies would be useful. Most oral studies of carbon tetrachloride toxicity in animals have involved administration of carbon tetrachloride by gavage in corn oil (Condie et al. 1986; Kim et al. 1990b). Since a bolus dose in oil may produce effects somewhat

different from those following intermittent exposure in water (e.g., greater hepatotoxicity when administered in oil, Condie et al. 1986), studies involving exposure in drinking water would be valuable, especially since this is a likely exposure pathway for residents using private wells near hazardous waste sites. More information on the mechanism of toxicity in tissues other than the liver (e.g., the kidney and nervous system) would be useful.

Chronic-Duration Exposure and Cancer. No definitive studies were located in humans on the noncarcinogenic effects of carbon tetrachloride after chronic-duration exposure. An occupational study by Tomenson et al. (1995) evaluated liver function, as indicated by the levels of hepatic enzymes in serum, in a cross-sectional study of individuals occupationally exposed to carbon tetrachloride. Although the exposed workers were categorized by their length of time on the job (<1 year, 1–5 years, and >5 years), this information was not included in the exposure-response analysis, so the effect of exposure duration is uncertain. A chronic inhalation MRL of 0.03 ppm was derived based on a NOAEL of 5 ppm (duration adjusted to 0.9 ppm) in a 2-year bioassay in rats (Japan Bioassay Research Center 1998; Nagano et al. 1998). Neither the rat nor the companion mouse inhalation bioassays reported definitive no-effect levels, but the target organs and effect levels were similar to those evident in subchronic assays. Cancer incidence in orally exposed animals was too high to make chronic exposure studies of noncarcinogenic effects practical or relevant. Therefore, no chronic oral MRL was derived.

The carcinogenicity of carbon tetrachloride was evaluated in rats and mice exposed intermittently by inhalation for 2 years (Japan Bioassay Research Center 1998; Nagano et al. 1998). These assays provided sufficient data for hepatic carcinogenicity in both sexes, and some evidence for a threshold effect in both species. The adrenal gland in mice was the only other tissue that had an increased tumor incidence. There is ample evidence that oral (Andervont 1958; Della Porta et al. 1961; Edwards 1941; Edwards et al. 1942; Eschenbrenner and Miller 1944, 1946; NCI 1976) and parenteral (Della Porta et al. 1961; Reuber and Glover 1967b, 1970) exposure to carbon tetrachloride can lead to increased tumor frequency in animals, but additional studies in which the chemical is administered in the food or drinking water would be helpful. Current oral data was derived from animals dosed by corn oil bolus gavage, a method of dosing that does not reflect human exposure calculations, and may yield artificial results as has been suggested by studies of other chlorinated methane and ethane compounds (Jorgenson et al. 1985; Kleming et al. 1986). While the carcinogenic risks of chronic dermal exposure have not been studied, chronic dermal exposure to carbon tetrachloride is not likely for most individuals.

Genotoxicity. Although it is evident that carbon tetrachloride exposure can increase the incidence of tumors in animals, it is not certain whether carbon tetrachloride is acting via a genotoxic mechanism, as a promoter, or both. Nearly all studies to date have failed to demonstrate any genotoxicity of carbon tetrachloride although lipid peroxidation products are genotoxic (Chaudhary et al. 1994; Chung et al. 2001; Wacker et al. 2001). Since it is believed that carbon tetrachloride toxicity is mediated at least in part through highly reactive and short-lived metabolites, further studies should focus particular attention on the issue of metabolic activation (especially anaerobic, reductive reactions), with *in vivo* or intact eukaryotic cell systems capable of activation *in situ* being preferred over systems relying on exogenous activation.

Reproductive Toxicity. The effects of carbon tetrachloride on reproduction have not been well investigated. Inhalation of carbon tetrachloride caused testicular degeneration (Adams et al. 1952) and reduced fertility (Smyth et al. 1936) in rats. Oral exposure to carbon tetrachloride did not adversely affect reproduction in rats (Alumot et al. 1976). Additional studies in animals using modern techniques and protocols for measuring adverse effects on reproductive parameters in males and females would be valuable. In order to be maximally useful, such studies should involve both oral and inhalation exposures, and should include a range of exposure levels extending below those that cause frank parental injury.

Developmental Toxicity. Epidemiological studies have been published on the developmental effects of carbon tetrachloride in humans (Bove et al. 1992a, 1992b, 1995; Croen et al. 1997). Limited data suggest that carbon tetrachloride has a low potential for developmental toxicity in animals. Fetal size was reduced and viability and lactation indices were decreased following inhalation exposures at or above 250 ppm (Gilman 1971; Schwetz et al. 1974). Fetotoxicity and teratogenicity were not seen in offspring coming to term, but total resorption of fetuses occurred in pregnant rats following oral exposure (Narotsky et al. 1997a, 1997b; Wilson 1954). Metabolic studies suggest that the fetuses of several rodent species, including the rat, lack the enzymes needed for activation of carbon tetrachloride, and that this may explain the low developmental toxicity. However, this phenomenon may not apply to humans, where some drug metabolizing activity develops *in utero*, especially in the developing brain (Brezinski et al. 1999). It would be useful to find nonrodent animal models, possibly primates, in which the MFO system also develops *in utero*, and use these to study the developmental toxicity of carbon tetrachloride. Studies are needed to evaluate the possible neurological or neurobehavioral effects of gestational exposure to carbon tetrachloride; parallel groups to evaluate the effect of maternal exposure to ethanol, which induces CYP2E1 would also be relevant to humans.

Immunotoxicity. There are a number of reports that parenteral exposure of animals to carbon tetrachloride can affect the immune system (Kaminski et al. 1989, 1990; Tajima et al. 1985). The effects of carbon tetrachloride on the immune system have been investigated following oral dosing (Smialowicz et al. 1991), but not after inhalation or dermal exposure. Studies of the immunotoxic potential of carbon tetrachloride by these routes would be valuable, especially in view of the scattered bits of suggestive data (McGuire 1932; Taylor 1925) indicating carbon tetrachloride may cause a hypersensitization reaction following dermal exposure. As noted by Luster et al. (1988), it is important that these studies include doses that do not cause systemic toxicity, so primary and secondary effects on the immune system can be distinguished.

Neurotoxicity. Available data make it clear that the central nervous system is a target organ for carbon tetrachloride, with the most obvious acute effects being central nervous system depression (Cohen 1957; Stevens and Forster 1953; Stewart et al. 1963). Although our understanding of this important aspect of carbon tetrachloride toxicity might benefit from further study of animals and accidentally exposed humans, of greater concern are the scattered reports that carbon tetrachloride exposure causes focal injury and degeneration of peripheral neurons. Additional studies by inhalation and oral routes would be helpful in defining the dose-response dependency of nerve cell injury, and in determining whether these effects are primary or are secondary to effects on the liver or kidneys.

Epidemiological and Human Dosimetry Studies. Several epidemiological studies have been conducted on the health effects of intermittent workplace exposure to carbon tetrachloride, primarily evaluating the effects on the central nervous system (Elkins 1942; Heimann and Ford 1941; Kazantzis and Bomford 1960), hepatic (Barnes and Jones 1967; Smyth et al. 1936; Tomenson et al. 1995), and renal (Barnes and Jones 1967) function in relatively small groups of workers. Cancer epidemiological studies have been conducted on significantly larger subject groups (Blair et al. 1998; Bond et al. 1986; Cantor et al 1985; Checkoway et al. 1984; Dumas et al. 2000; Heineman et al. 194; Kernan et al. 1999; Wilcosky et al. 1984). Epidemiological studies evaluated developmental effects (Bove et al. 1992a, 1992b, 1995; Croen et al. 1997) in populations exposed to carbon tetrachloride in drinking water, which is a route of exposure that may be of concern near hazardous waste sites. A common problem in epidemiological studies is the acquisition of reliable dosimetry data on the exposed populations. For this reason, efforts to improve estimates of past exposures and to define more accurately current exposure levels to carbon tetrachloride would be valuable.

Biomarkers of Exposure and Effect. The presence of carbon tetrachloride in expired air is the most commonly used biomarker of exposure. The rate of excretion in humans appears to be biphasic, with an initial elimination half-life of less than 1 hour, and a second phase of about 30–40 hours. The compound can be detected in expired air within hours to weeks after exposure. Research on additional biomarkers of exposure would be of value, perhaps in areas such as detection of DNA adducts.

There are a number of clinical and biochemical tests available that can detect early signs of hepatic and renal injury in humans. However, these tests are not specific for carbon tetrachloride-induced effects. For this reason, studies to identify and measure effects more diagnostic of carbon tetrachloride-specific injury would be helpful. Also, improvements in the sensitivity of these tests, such as accomplished by Ikemoto et al. (2001), would be valuable in evaluating the health status of individuals who have been exposed to low levels of carbon tetrachloride.

Absorption, Distribution, Metabolism, and Excretion. There is relatively little quantitative information on the systemic absorption of inhaled carbon tetrachloride in animals and humans, with estimates ranging from 30 to 60% (Lehmann and Schmidt-Kehl 1936; McCollister et al. 1951). Sanzgiri et al. (1995, 1997) have compared uptake, distribution, and elimination of carbon tetrachloride administered to rats over 2 hours by inhalation or gastric infusion or as a single bolus by gavage and correlated the results with the severity of hepatic injury. This study provides information pertinent to a route-to-route extrapolation.

Although dermal absorption of carbon tetrachloride is relatively modest compared to absorption by the oral or inhalation routes, it would be helpful to quantify the rate and extent of percutaneous absorption of carbon tetrachloride from water. This information would be useful in determining the contribution of dermal exposure to the total dose received by persons using carbon tetrachloride-contaminated drinking water for bathing or showering, or to those who contact carbon tetrachloride-contaminated water near chemical waste sites.

Animal studies reveal that carbon tetrachloride is distributed to tissues according to their rate of blood perfusion and lipid content. Adipose tissue accumulates much higher concentrations of carbon tetrachloride than other tissues, due to the high oil:water partition coefficient of carbon tetrachloride. The animal tissue distribution data are limited, in that carbon tetrachloride levels in tissues in rats have been determined at only a few time-points after a single, high oral dose (Marchand et al. 1970; Teschke

et al. 1983). Paustenbach et al. (1986a, 1986b) have measured ^{14}C-carbon tetrachloride levels in tissues of rats at just one time-point following repeated inhalation exposure regimens.

Comparative Toxicokinetics. Metabolic pathways and mechanisms of hepatotoxicity of carbon tetrachloride have been the subject of many studies in intact animals and *in vitro*, and are therefore better understood than for many other chemicals. However, there are apparently little data on metabolism of carbon tetrachloride in humans. It would be valuable to conduct *in vitro* experiments with human liver samples and hepatocytes to determine whether metabolic pathways and toxic metabolites are similar to those found in animals. It would also be beneficial to identify an animal model in which MFO systems develop in utero as they do in the human fetus.

PBPK models have been developed for a number of drugs and chemicals, in order to better understand and simulate the dynamics of those compounds in the body. Advances made to date indicate that valid PBPK models can accurately predict the concentration of chemicals over time in the blood and specific tissues. Blood and tissue concentration versus time profiles, as well as excretion patterns from animals have been used to validate and adjust PBPK models for carbon tetrachloride (Gallo et al. 1993; Paustenbach et al. 1988). Addition of parameter values for humans has been used to scale-up the PBPK model to predict target tissue uptake, metabolism, and elimination of carbon tetrachloride in humans (Thrall et al. 2000). Quantitative relationships between carbon tetrachloride levels in target organs and organ damage in animals could be used to establish toxicodynamic models. Accurate prediction of ultimate toxicological outcomes will likely also have to account for base-line and inducible levels of compensatory repair mechanisms. Combined PBPK-toxicodynamic models might then be scaled up and used to predict target organ concentrations and toxicity of carbon tetrachloride in man.

Methods for Reducing Toxic Effects. The usefulness of methods and treatments for reducing peak absorption and reducing the body burden of carbon tetrachloride is rather limited due to the chemical's rapid rates of absorption and tissue disposition. On the other hand, investigations of antidotal therapy based on the mechanism of action have been limited to a few studies involving the administration of compounds to reduce free radical injury. Additional studies would be useful to better establish the effectiveness of both acute and prolonged antidotal therapy, since carbon tetrachloride is persistent in the body.

Children's Susceptibility. The difference between the toxicity of carbon tetrachloride in children and adults is likely to be dependent on the relative expression of CYP2E1. Viera et al. (1996) determined

that hepatic levels of CYP2E1 in children reach adult levels sometime between the ages of 1 and 10. Additional studies are needed to obtain a precise chronology of the increase. Furthermore, additional studies are needed to clarify fetal expression of CYP2E1 to determine the sensitivity of different fetal tissues and the placenta during gestation.

Child health data needs relating to exposure are discussed in 6.8.1 Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Numerous current publications on carbon tetrachloride have addressed the efficacy of various agents for reducing or eliminating the toxic effects of exposure; these are mentioned in Section 3.11.3. Additional research programs are focusing on potential therapeutic agents, interacting factors, or mechanisms of toxicity following exposure to carbon tetrachloride.

In a Small Business Innovation Research study funded by the Department of Health and Human Services, J.W. Larrick of Panorama Research, Inc. is engaged in cloning the gene for fetal hepatopoietin (hepatocyte growth factor). This protein has been shown to be protective in mice against hepatic injury caused by carbon tetrachloride. The long-range goal of the study is to investigate the diagnostic and therapeutic potential of the protein with respect to a variety of hepatic diseases.

In a study supported by the Department of Agriculture, R.A. Disilvestro of Ohio State University is evaluating the effect of different dietary levels of copper and zinc in rats on the accumulation of free radicals following oxidative stress caused by exposure to carbon tetrachloride. The study has been extended to cover humans and dogs.

Dr. T.R. Morgan, of the Department of Veterans Affairs, is using a transgenic mouse model to determine whether over-expression of human CYP2E1 increases the susceptibility to liver injury following acute exposure to carbon tetrachloride. These studies are to be conducted along with other studies evaluating the physiology of alcoholic liver damage.